Sex Differences in Cardiopulmonary Responses to Exercise

by

JOSHUA RICHARD SMITH

B.S., Indiana University, 2011
M.S., Kansas State University, 2013

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Kinesiology
College of Human Ecology

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2017

Abstract

The overall aim of this dissertation is to further understand sex differences in the cardiopulmonary responses during exercise in younger and older individuals. Emphasis is directed towards the influence of sex in modulating respiratory muscle blood flow and the inspiratory muscle metaboreflex. The first investigation of this dissertation (Chapter 2) demonstrated that sex differences do not alter respiratory muscle blood flow at rest or during exercise. The second investigation (Chapter 3) demonstrated that sex differences exist in the cardiovascular consequences of the inspiratory muscle metaboreflex. Specifically, pre-menopausal women, compared to age-matched men, exhibited attenuated increases in mean arterial pressure and limb vascular resistance as well as decreases in limb blood flow during inspiratory muscle metaboreflex activation. In Chapter 4, we demonstrated that post-menopausal, compared to pre-menopausal, women exhibit greater increases in mean arterial pressure and limb vascular resistance and decreases in limb blood flow during activation of the inspiratory muscle metaboreflex. Furthermore, no differences in the cardiovascular consequences were present between older men and women or younger and older men with activation of the inspiratory muscle metaboreflex. These data suggest that the tonically active inspiratory muscle metaboreflex present during maximal exercise will result in less blood flow redistribution away from the locomotor muscles in pre-menopausal women compared to post-menopausal women, as well as younger and older men. In conclusion, sex differences in young adults incur a major impact in the cardiovascular consequences during inspiratory muscle metaboreflex activation, while not modifying respiratory muscle blood flow.

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Approved by:

Major Professor
Dr. Craig A. Harms

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The overall aim of this dissertation is to further understand sex differences in the cardiopulmonary responses during exercise in younger and older individuals. Emphasis is directed towards the influence of sex in modulating respiratory muscle blood flow and the inspiratory muscle metaboreflex. The first investigation of this dissertation (Chapter 2) demonstrated that sex differences do not alter respiratory muscle blood flow at rest or during exercise. The second investigation (Chapter 3) demonstrated that sex differences exist in the cardiovascular consequences of the inspiratory muscle metaboreflex. Specifically, pre-menopausal women, compared to age-matched men, exhibited attenuated increases in mean arterial pressure and limb vascular resistance as well as decreases in limb blood flow during inspiratory muscle metaboreflex activation. In Chapter 4, we demonstrated that post-menopausal, compared to pre-menopausal, women exhibit greater increases in mean arterial pressure and limb vascular resistance and decreases in limb blood flow during activation of the inspiratory muscle metaboreflex. Furthermore, no differences in the cardiovascular consequences were present between older men and women or younger and older men with activation of the inspiratory muscle metaboreflex. These data suggest that the tonically active inspiratory muscle metaboreflex present during maximal exercise will result in less blood flow redistribution away from the locomotor muscles in pre-menopausal women compared to post-menopausal women, as well as younger and older men. In conclusion, sex differences in young adults incur a major impact in the cardiovascular consequences during inspiratory muscle metaboreflex activation, while not modifying respiratory muscle blood flow.

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Preface

Chapters 2-4 of this dissertation represent original research articles that have been published following the peer-review process (citations may be found below). They are reproduced here with permission from the publishers.

Smith JR, Hageman KS, Harms CA, Poole DC, Musch TI. Respiratory muscle blood flow during exercise: effects of sex and ovarian cycle. *Journal of Applied Physiology* In press, doi:10.1152/japplphysiol.01007.2016

Smith JR, Broxterman RM, Hammer SM, Alexander AM, Didier KD, Kurti SP, Barstow TJ, Harms CA. Sex differences in the cardiovascular consequences of the inspiratory muscle metaboreflex. *American Journal of Physiology-Regulatory, Integrative, and Comparative Physiology* 311(3): R574-R581, 2016.

Smith JR, Alexander AM, Hammer SM, Dider KD, Kurti SP, Broxterman RM, Barstow TJ, Harms CA. Cardiovascular consequences of the inspiratory muscle metaboreflex: effects of age and sex. *American Journal of Physiology-Heart and Circulatory Physiology* doi:10.1152/ajpheart.00818.2016.

Chapter 1 - Introduction

The respiratory muscles are structurally (high mitochondrial volume density, rich vascular network) and functionally (great oxidative potential and vascular flow capacity) designed to resist fatigue and failure (2, 17-19, 20, 21). From rest to maximal exercise, the cost and work of breathing increases with progressive increases in ventilation during incremental exercise, which are met by increases in respiratory muscle blood flow (15). During exercise, the respiratory muscle blood flow response is heterogenous, such that diaphragm blood flow increases to a greater extent than accessory respiratory muscle (e.g., intercostal, transversus abdominis) blood flow (20). The high ventilatory demand and work of breathing during maximal exercise commands that 14-16% of the total cardiac output be distributed to the respiratory muscles (10).

Sex differences exist in airway caliber and respiratory mechanics during exercise. For example, women have smaller airways compared to men matched for lung size (24), which is associated with the development of expiratory flow limitation during exercise (25). Consequently, women have a higher cost and work of breathing for a given ventilation during exercise (4, 7). These latter studies suggest that women compared to men will have a greater respiratory muscle blood flow response during exercise. In this context, we developed the investigation described in Chapter 2 to examine if sex differences exist in respiratory muscle (diaphragm, intercostals, and transversus abdominis) blood flow during moderate and near-maximal intensity exercise.

High inspiratory muscle work and the concomitant accumulation of metabolites is associated with neural and cardiovascular consequences (3). Specifically, fatiguing diaphragmatic contractions lead to increased group IV afferent discharge (12) and stimulation of

the phrenic afferents leads to vasoconstriction and decreased blood flow to the periphery (13). Furthermore, lactic acid infusion into the phrenic artery resulted in increased mean arterial pressure (MAP) and decreased limb blood flow ($\dot{Q}_L$) at rest and during exercise in canines (22). In young men, high inspiratory muscle work activated the inspiratory muscle metaboreflex leading to time-dependent increases in muscle sympathetic nerve activity (MSNA), MAP, and leg vascular resistance (LVR), such that the increase in LVR was greater than MAP resulting in decreased $\dot{Q}_L$ (23, 26). There is also evidence that the inspiratory muscle metaboreflex is tonically-active during severe-intensity exercise in young men. Harms et al (1997) found that unloading the inspiratory muscles (via a proportional assist ventilator) during maximal cycling exercise decreased LVR by ~7% compared to control (9). Therefore, the inspiratory muscle metaboreflex leads to neural and cardiovascular consequences during maximal exercise.

Due to the greater cost and work of breathing in women compared to men (4, 7), it has been suggested that women have an exaggerated inspiratory muscle metaboreflex, consequently leading to greater redistribution away from the exercising locomotor muscles. However, sex differences have also been found in the skeletal muscle metaboreflex-induced neural and cardiovascular responses (5, 14). Specifically, the skeletal muscle metaboreflex elicits attenuated increases in MSNA and MAP in women compared to men (5, 14). In addition, sex differences exist in the peripheral transduction of sympathetic outflow to the peripheral vasculature (11). In Chapter 3, we determined if sex differences exist in the cardiovascular consequences of the inspiratory muscle metaboreflex. Identifying sex differences in the inspiratory muscle metaboreflex represents the initial step towards determining if high inspiratory muscle work will lead to sex differences in the redistribution of locomotor $\dot{Q}_L$ during maximal exercise.

Based on these data, we investigated if the cardiovascular consequences of the inspiratory muscle metaboreflex were modified in older adults (Chapter 4). Aging is associated with decreases in respiratory muscle strength (8) and endurance (1), loss of elastic recoil (16) and stiffening of the chest wall (15). Consequently, dynamic compliance is lower in older adults, such that the cost and work of breathing for a given ventilation is higher compared to younger adults (15). Moreover, post-menopausal women compared to pre-menopausal women demonstrate greater sympathetic vasoconstriction during exercise (6) and more transduction of sympathetic outflow to the peripheral vasculature (11). In this context, we developed the investigation in Chapter 4 to determine the influence of age on sex differences in the cardiovascular consequences of the inspiratory muscle metaboreflex.

Taken together, the studies included in this dissertation were designed to extend the existing literature by examining the roles of sex and age on respiratory muscle blood flow as well as the inspiratory muscle metaboreflex. Significant focus is given to sex differences in the inspiratory muscle metaboreflex and if these sex differences are present in older adults. Each chapter in this dissertation is self-contained following standard journal article format and a comprehensive conclusion is included for this series of studies (Chapter 5).

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Chapter 2 - Respiratory muscle blood flow during exercise: effects of sex and ovarian cycle

Summary

Sex and ovarian cycle have been speculated to modify respiratory muscle(s) blood flow control during exercise, but the findings are inconclusive. We tested the hypotheses that females would have higher respiratory muscle blood flow and vascular conductance (VC) compared to males during exercise and that this difference would be accentuated in proestrus versus ovariectomized (OVA) females. Mean arterial pressure (carotid artery catheter) and respiratory muscle blood flow (radiolabeled microspheres) were measured during moderate-intensity (24m/min, 10% grade) exercise in male (n=9), female (n=9), and OVA female (n=7) rats and near-maximal (60m/min, 5% grade) exercise in male (n=5) and female (n=7) rats. At rest, diaphragm, intercostal, and transversus abdominis blood flow were not different ($p=0.33$) among groups. During moderate-intensity exercise, diaphragm (M: 124±16; F: 140±14; OVA: 140±20mL/min/100g), intercostal (M: 33±5; F: 34±5; OVA: 30±5mL/min/100g), and transversus abdominis blood flow (M: 24±4; F: 35±7; OVA: 35±9mL/min/100g) significantly increased in all groups compared to rest, but were not different ($p=0.12$) among groups. From rest to moderate-intensity exercise, diaphragm ($p<0.03$) and transversus abdominis ($p<0.04$) VC increased in all groups, while intercostal VC increased only for males and females ($p=0.01$). No differences ($p>0.13$) existed in VC among groups. During near-maximal exercise, diaphragm (M: 304±62; F: 283±17mL/min/100g), intercostal (M: 29±8; F: 40±6mL/min/100g), and transversus abdominis (M: 85±14; F: 86±9mL/min/100g) blood flow and VC were not different ($p>0.27$) between males and females. These data demonstrate that respiratory muscle blood flow and vascular conductance at rest and during exercise are not affected by sex or ovarian cycle in rats.

Introduction

Respiratory muscle failure portends morbidity and mortality and thus the respiratory muscles are structurally (high mitochondrial volume density, rich vascular network) and functionally (great oxidative potential and vascular flow capacity) designed to resist fatigue and failure (7, 32-34, 47, 48). A key aspect of respiratory muscle function during exercise is that, as befitting their importance to overall exercise capacity, increased respiratory muscle work can command a redistribution of cardiac output from the locomotory to the respiratory muscles in healthy (20) and diseased (37, 43) populations.

Although not possible in humans, respiratory muscle blood flow has been directly measured in rats (37, 47), dogs (39), swine (2), and ponies (32-34). From rest to maximal exercise, blood flow increases heterogeneously to the respiratory muscles, with greater diaphragm than intercostal and transversus abdominis blood flow (34, 37, 47). Whereas diaphragm paralysis decreases peak oxygen uptake ~20% in rats (18), the large increases in its blood flow during exercise suggest that the diaphragm is an important inspiratory muscle in rats as for humans.

Accumulating evidence suggests that sex differences in respiratory muscle blood flow during exercise may exist. Specifically, women have been shown to have a higher cost of breathing compared to men at submaximal and maximal ventilations (8). In addition, hyperthermia-induced increases in ventilation lead to greater increases in diaphragm blood flow in resting female rabbits compared to male rabbits (31). These data suggest that females will have higher respiratory muscle blood flow than males during exercise; however, this has not been investigated.

Several studies have investigated if sex differences exist in the regulation of limb muscle blood flow during exercise; however, the results are inconclusive. For example, pre-menopausal women have been reported to have higher forearm and knee extensor muscle blood flow and vascular conductance compared to men during handgrip (17, 28, 51) and knee extension exercise (39), respectively. In contrast, several other studies have found no sex differences in muscle blood flow and vascular conductance during handgrip exercise (5, 25, 29, 30, 58).

Animal models allow for experimental designs that can mechanistically elucidate differences in blood flow regulation between sexes. Rogers and Sheriff (2004) reported that sex differences were not present in terminal aortic blood flow during treadmill running in rats. However, chronically supplementing ovariectomized female rats with estrogen resulted in greater terminal aortic blood flow and vascular conductance during exercise compared to males (50). Thus, females tested during the proestrus stage of their ovarian cycle may exhibit greater respiratory muscle blood flow and vascular conductance during exercise compared to males. Therefore, the effects of sex and the ovarian cycle on respiratory muscle blood flow regulation and vascular conductance during exercise remain unresolved. We hypothesized that females would exhibit greater respiratory muscle blood flow and vascular conductance compared to males during exercise and that these differences would be accentuated in proestrus versus ovariectomized females.

Methods

Ethical approval: Age-matched male (n=14; 388-421 g), female (n=16; 263-291 g), and OVA female (n=7; 355-436 g) Wistar rats (Charles River Laboratories, Portage MI) were maintained at accredited animal facilities at Kansas State University on a 12:12-h light-dark cycle with food and water provided *ad libitum*. All procedures were approved by the Institutional Animal Care and Use Committee of Kansas State University. Vaginal smear cytology (35) was used to confirm females were tested in the proestrous phase and the OVA females were devoid of the ovarian cycle. All rats were familiarized with running on a custom-built, motor-driven treadmill for two weeks consisting of exercising 5-10 min/day at 20m/min, 10% grade. The speed of the treadmill was gradually increased to 28 m/min while exercise duration was reduced to <5 min/day to ensure a training effect was not incurred (42).

Surgical instrumentation: The ovariectomy procedure consisted of administration of anesthesia (5% halothane oxygen mixture) followed by bilateral midventral incisions to locate the ovaries. The junction between the uterus and each ovary was ligated followed by ovary resection, closure of incisions, and a minimum 4 weeks of recovery. On the day of the experiment, rats were anesthetized with a 5% halothane oxygen mixture and maintained subsequently on a 2% halothane/oxygen combination. One catheter (PE-10 connected to PE-50, IntraMedic polyethylene tubing, Clay Adams, Becton, Dickinson, Sparks, MD) was placed in the ascending aorta via the right carotid artery for the measurement of mean arterial pressure (MAP) and heart rate (HR) (model 200, DigiMed BPA, Louisville, KY) and the infusion of radiolabeled microspheres. A second catheter was placed in the caudal (tail) artery for arterial blood sampling (42). Both catheters were tunneled subcutaneously through the dorsal aspect of the cervical

region and exteriorized through a puncture in the skin. The incisions were then closed, anesthesia was terminated, and the rats were given >60 min to recover (13).

Measurement of respiratory muscle blood flow: Following recovery, each rat was placed on the motor-driven treadmill and the carotid artery catheter was connected to the pressure transducer (model P23ID, Gould Statham, Valley View, OH). After the stabilization period, the caudal artery was connected to a 1-mL syringe chambered in an infusion/withdrawal pump (model 907, Harvard Instruments). Rats were given a 2-min warm-up period followed by a gradual increase in grade and speed of the treadmill to 24 m/min, 10% grade (~60%$\dot{V}O_2$max, moderate-intensity exercise) for males (n=9), females (n=9), and OVA females (n=7) or 60 m/min, 5% grade (near-maximal exercise) for males (n=5) and females (n=7). The rat was then required to exercise at the speed and grade for 3 min. During this time, radiolabeled microspheres (^{46}Sc, ^{85}Sr, ^{141}Ce, or ^{113}Sn in random order; New England Nuclear, Boston, MA) were mixed by a vortex agitator (Fishers Scientific, Waltham, MA). At the 3 min exercise mark, the carotid artery catheter was disconnected from the pressure transducer and 0.5-0.6 x 10^6 microspheres with a 15-μm diameter were injected into the aortic arch to determine respiratory muscle blood flow. Simultaneously, the pump connected to the caudal artery catheter was activated and blood withdrawal was initiated at a rate of 0.25 mL/min. Blood withdrawal was terminated 30 s following the microsphere infusion and then exercise was terminated.

After >30 min of recovery, a second microsphere infusion was performed (radiolabeled different from the first microsphere infusion) while the rat sat quietly on the treadmill for determination of resting respiratory muscle blood flows, MAP and HR. This experimental protocol minimizes the potential influences of the pre-exercise anticipatory response on resting muscle blood flows, MAP and HR measurements (3).

Determination of blood flow and vascular conductance: Following the completion of the exercise protocols, rats were euthanized with an overdose of sodium pentobarbital (>50 mg/kg body wt.) via the right carotid artery catheter and placement of each catheter was verified by anatomic dissection. The diaphragm, intercostals, and transversus abdominis of each rat were dissected out. The tissues were blotted, weighed, and placed immediately into counting vials. Tissue blood flows were determined using the radionuclide-tagged microsphere technique that has previously been used in the exercising rat (42). Before each injection, the microspheres were thoroughly mixed and agitated by sonication to prevent clumping. Each group of microspheres (0.6-0.7 x 10^6 in number) was injected into the ascending aorta of the rat in a 0.15-0.20 mL volume. The radioactivity of each tissue was determined with a gamma scintillation counter (model 5230, Auto Gamma Spectrometer, Packard, Downers Grove, IL). The radioactivity of the tissues was then analyzed by computer, taking into account the cross-talk fraction between the different isotopes. Absolute muscle blood flow was then calculated by the reference sample method (26) and expressed in milliliters per min per 100g of tissue. Vascular conductance was then calculated by normalizing blood flow to MAP measured at the time of the microsphere infusion and expressed as mL/min/mmHg/100g.

Statistical analyses: Values are reported as mean ± standard error (SE). All statistical analyses were performed by using SigmaStat 2.0 (Jandel Scientific, San Rafael, CA). MAP, HR as well as respiratory muscle (diaphragm, intercostal, and transversus abdominis) blood flows and vascular conductances were compared within (rest vs 24 m/min, 10% grade) and among (males vs females vs OVA females) groups using mixed factorial ANOVAs and Student-Newman-Keuls post-hoc tests when appropriate. Unpaired *t*-tests were used to compare MAP, HR, and respiratory muscle (diaphragm, intercostal, and transversus abdominis) blood flows and vascular

conductances between males and females. Two-tailed p values were reported and statistical significance was set at $p<0.05$.

Results

Cardiovascular responses: At rest, there were no significant differences in MAP (M: 137±5; F: 131±4; OVA: 140±5 mmHg; p=0.23) or HR (M: 453±9; F: 439±16; OVA: 429±19 beats/min; p=0.35) between groups (Table 1). During moderate-intensity exercise, HR significantly increased (p<0.01) in each group, but was not different (p=0.12) between groups (M: 536±5; F: 544±13; OVA: 509±6 beats/min). During moderate-intensity exercise, MAP was not different (p=0.08) compared to resting values or among groups (p=0.38). During near-maximal exercise, MAP was not significantly different (p=0.09) between males (137±4 mmHg) and females (145±2 mmHg), but HR was significantly higher (p<0.01) for females (550±5 beats/min) compared to males (514±5 beats/min).

Respiratory muscle blood flow: At rest, diaphragm (Fig. 1A), intercostal (Fig. 1B), and transversus abdominis (Fig. 1C) blood flows were not different (p=0.33) among groups. From rest to moderate-intensity exercise, diaphragm, intercostal, and transversus abdominis blood flow significantly increased in males, females, and OVA females, but no differences (p=0.12) existed among groups. Individual male and female diaphragm blood flow responses from rest to moderate-intensity exercise are shown in Figure 3A. No differences were present in resting diaphragm (M: 0.60±0.07; F: 0.58±0.06; OVA: 0.73±0.06 mL/min/mmHg/100g; p=0.38), intercostal (M: 0.12±0.03; F: 0.09±0.02; OVA: 0.14±0.04 mL/min/mmHg/100g; p=0.42), or transversus abdominis (M: 0.08±0.01; F: 0.09±0.02; OVA: 0.12±0.02 mL/min/mmHg/100g; p=0.53) vascular conductance among males, females, and OVA females. From rest to moderate-intensity exercise, diaphragm (M: 0.87±0.11; F: 1.02±0.10; OVA: 0.99±0.13 mL/min/mmHg/100g; p<0.03) and transversus abdominis (M: 0.17±0.03; F: 0.25±0.05; OVA: 0.25±0.06 mL/min/mmHg/100g; p<0.04) vascular conductance increased in all groups, while

intercostal vascular conductance significantly increased only for males (0.23±0.04 mL/min/mmHg/100g; $p=0.01$) and females (0.25±0.04 mL/min/mmHg/100g; $p<0.01$). There were no significant differences among groups in diaphragm ($p=0.39$), intercostal ($p=0.67$), or transversus abdominis vascular ($p=0.13$) conductance during moderate-intensity exercise.

Similarly, there were no sex differences in diaphragm ($p=0.71$), intercostal ($p=0.27$), or transversus abdominis ($p=0.95$) muscle blood flow (Fig. 2A) during near-maximal exercise. Figure 3B shows individual male and female diaphragm blood flows during near-maximal exercise. There were also no sex differences in diaphragm ($p=0.52$), intercostal ($p=0.42$), or transversus abdominis ($p=0.88$) vascular conductance (Fig. 2B) during near-maximal exercise.

Discussion

Major findings: The primary novel findings of this investigation are that respiratory muscle blood flow and vascular conductance were neither significantly different between (1) male and female rats at rest or during moderate- or near-maximal-intensity exercise nor (2) females tested during the proestrus phase and ovariectomized females during moderate-intensity exercise. These results suggest that respiratory muscle blood flow responses and vascular conductance during exercise are not modulated by sex or the ovarian cycle in rats.

Sex differences in respiratory muscle blood flow: Blood flow control differs between men and women. For example, women exhibit attenuated increases in muscle sympathetic nerve activity during exercise (9, 27) as well as less peripheral transduction of sympathetic outflow to the peripheral vasculature than men (23). Moreover, elevated estrogen levels as seen during the proestrus phase lead to greater synthesis of prostacyclin and nitric oxide (24, 44) coincident with greater endothelium-dependent vasodilation (21). These data suggest women may have greater exercise-induced muscle vasodilation and blood flow responses compared to men.

The literature investigating sex differences in blood flow during exercise is equivocal. For example, Parker et al (2007) found that women had higher femoral artery blood flow (normalized for muscle mass) compared to men during knee extension exercise (46). In contrast, several studies report no sex differences in blood flow responses during handgrip exercise (5, 17, 25, 29, 30, 58). Furthermore, Rogers and Sheriff (2004) could found no sex differences in terminal aortic blood flow during treadmill exercise in rats. However, ovariectomized female rats with chronic estrogen supplementation had higher terminal aortic blood flow and vascular conductance compared to males. Therefore, we reasoned that females tested during the proestrus stage of their ovarian cycle would have higher exercising respiratory muscle blood flow and

vascular conductance compared to males. In contrast, we found no sex differences in respiratory muscle blood flow, vascular conductance, or heterogeneity in blood flow among respiratory muscles during moderate-intensity or near-maximal exercise. Importantly, our near-maximal exercise respiratory muscle blood flows, specifically diaphragm blood flow (i.e., 283-304 mL/min/100g), are in line with maximal-intensity exercise values previously reported in ponies (265-325 mL/min/100g; 32, 34), while lower than those reported in rats exercising supra-maximally (360 mL/min/100g; 47) and maximal-intensity human knee-extensor blood flow (385 mL/min/100g; 49). In addition, the heterogeneity in respiratory muscle blood flow and vascular conductances during exercise is consistent with previous studies in male (37) and female (47) rats as well as ponies (34). A likely explanation for the discrepancy between our findings and those using the chronic estrogen supplementation stated above (50) stems from their implementation of supra-physiologic estrogen levels. Furthermore, our results are in-line with studies showing no differences in exercising brachial artery blood flow between men and women tested in the late follicular or luteal phases (30, 58). This may reflect a substantial redundancy of blood flow control mechanisms that regulate sex differences at rest and during exercise.

The findings of the current investigation also contribute to our understanding of the redistribution of blood flow from the locomotory muscles to the respiratory muscles during severe-intensity exercise. Specifically, high inspiratory muscle work and concomitant accumulation of metabolites lead to an inspiratory muscle metaboreflex (52, 55) redistributing blood flow to the respiratory from the locomotory muscles during exercise (20). Recently, we have found that women have an attenuated inspiratory muscle metaboreflex (53) and less exercise-induced diaphragmatic fatigue development compared to men (19). Currently, it is not possible to measure diaphragm blood flow directly in humans; however, it has been suggested

that women have a greater portion of cardiac output directed to the inspiratory muscles (8). In the current study, we found that sex differences were not present in diaphragm blood flow or vascular conductance during near-maximal running. It is important to note that respiratory mechanics differ between rats and humans. At rest, rats have greater lung and chest wall compliance as well as less lung resistance compared to humans (6). It remains to be determined whether altering the work of breathing via stimuli other than exercise or in addition to exercise exposes sex differences in cardiac output redistribution during exercise.

Ovarian cycle and respiratory muscle blood flow: Previous studies investigating the effects of the ovarian cycle on neural and blood flow control have been conflicting. While not a consistent finding (27), muscle sympathetic nerve activity has been reported to be higher during menses compared to late follicular phase in women during handgrip exercise (10). In addition, endothelium-dependent flow mediated dilation is also greater during late follicular and luteal phase compared to menses possibly due to the high circulating estrogen levels (21).

To date, few investigations have studied the influence of the ovarian cycle on muscle blood flow responses during exercise. A recent study reported that brachial artery blood flow and vascular conductance were not altered across the menstrual cycle during dynamic handgrip exercise at 15% and 30%MVC (30). In the present study, we found that respiratory muscle(s) blood flow and vascular conductance were not different between ovariectomized females and females tested in the proestrus phase. To our knowledge, this is the first study to compare muscle blood flows during large muscle mass exercise (i.e. running) in ovariectomized females and females tested in the proestrus phase.

In contrast to our findings, chronic estrogen supplementation leads to higher resting femoral artery blood flow and vascular conductance (36) and attenuated limb vasoconstriction in

post-menopausal women (11) as well as increased blood flow and vascular conductance during exercise in ovariectomized female rats (12, 50). Furthermore, estrogen has been shown to upregulate nitric oxide (22, 56), suppress α1-aderngergic receptor expression (59) and decrease sympathetic innervation (60). While not a consistent finding (15), it is possible the high progesterone levels, which also occur during the proestrus phase, may have attenuated the vasodilatory effects of estrogen in the current study (14). Future studies are warranted to elucidate the mechanisms responsible for the divergent effect of the ovarian cycle versus chronic estrogen supplementation on blood flow responses during exercise. Collectively, our findings in addition to previous studies suggest that the ovarian cycle does not modulate conduit artery or muscle blood flow during whole-body exercise.

Experimental considerations: We recognize that the lack of respiratory muscle blood flow differences either between male and female rats or between intact and OVA rats at rest and during exercise may conceal disparities in respiratory mechanics, muscle recruitment, and/or work and control of breathing. Moreover, demonstration that there are no male/female differences in $\dot{V}O_2$, $\dot{V}CO_2$, or $PaCO_2$ between male and female rats at moderate and near-maximal-intensity exercise would have helped alleviate these concerns. In this regard, the literature demonstrates that, despite significant differences in body mass with females being smaller (herein by 124 g on average), female and male rats during treadmill exercise do not differ with respect to mass-specific $\dot{V}O_2$ or $\dot{V}CO_2$ (4, 16, 38, 40, 41). Moreover, because there are no differences in $PaCO_2$ (38, 40, 41), alveolar minute ventilation is not expected to be different between males and females. While we recognize that sex-related differences in airway size and dead-space as well as respiratory mechanics and thus work of breathing may not be the same, we consider it unlikely that these considerations would coincidentally summate with

alterations of respiratory muscle vascular control to nullify differences in vascular conductance and blood flow in the respiratory muscles should they exist.

With respect to females being lighter than males; whereas across orders of body mass larger animals move more efficiently (e.g., lower $\dot{V}O_2$ or J per m per kg (1)), within a given species the literature supports that there is an invariance of body mass-adjusted $\dot{V}O_2$ during rest or running among both animals (1) and humans (57). Furthermore, the energetic cost of running was not different between male and female middle-distance (1500-3000 m) runners (45). Finally, whereas it is obvious that locomotory energetics differ between bipedal and quadrupedal running, the metabolic cost per kg lifted is not different in bipeds versus quadrupeds (54).

Conclusion: This is the first investigation to resolve that there is no influence of sex and ovarian cycle on respiratory muscle blood flow and vascular conductance at rest and during exercise in rats. However, this does not address the important question as to whether altering the work of breathing during exercise might expose sex differences in respiratory muscle blood flow and if progesterone is attenuating the vasodilatory effects of estrogen during exercise. It would also be pertinent to determine if sex differences are present in hindlimb blood flow and vascular conductance during exercise.

Table 2-1 MAP and HR at rest and during moderate-intensity exercise.

	MAP (mmHg)	HR (beats/min)
Male		
Rest	137 ± 5	453 ± 9
Exercise	142 ± 2	536 ± 5*
Female		
Rest	131 ± 4	439 ± 16
Exercise	137 ± 4	544 ± 13*
OVA Female		
Rest	140 ± 5	429 ± 19
Exercise	142 ± 3	509 ± 6*

Values are mean ± SE. MAP, mean arterial pressure; HR, heart rate. * $p<0.05$ vs. rest

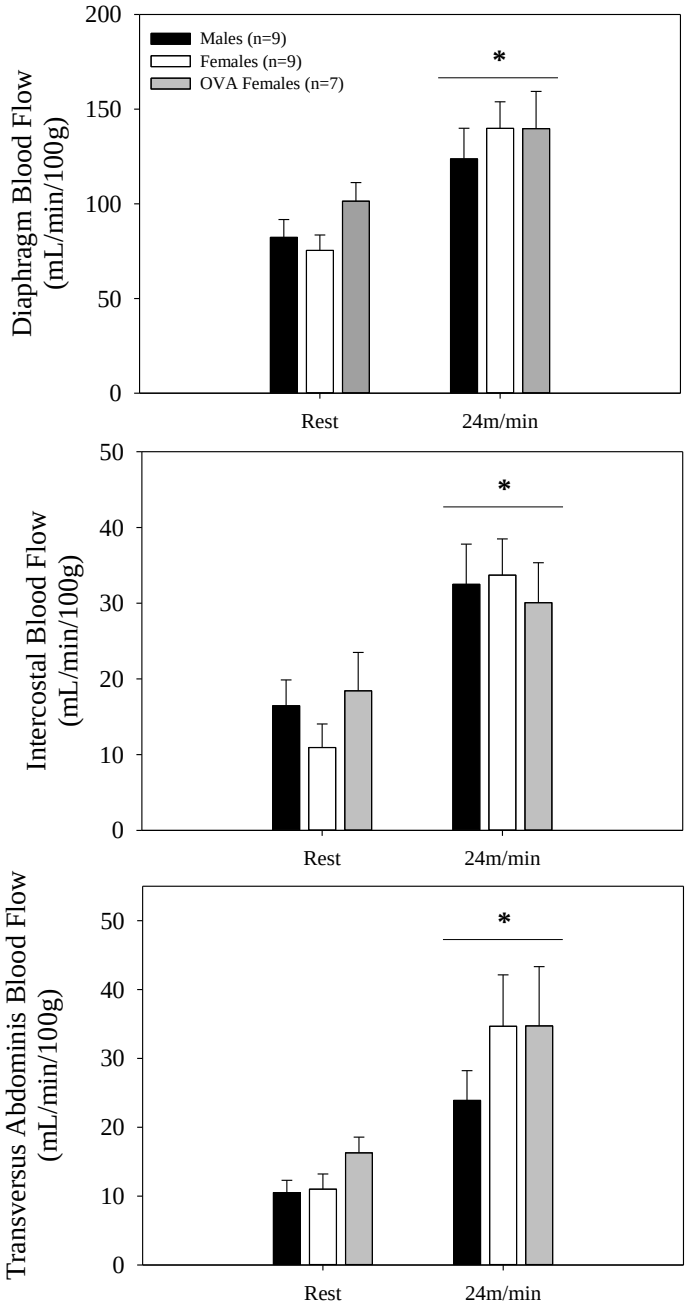

Figure 2-1 Respiratory muscle blood flows at rest and during moderate-intensity exercise. Diaphragm (top), intercostal (middle), and transversus abdominis (bottom) blood flow at rest and during moderate-intensity exercise in males (black bar), females (white bar), OVA females (gray bar). Diaphragm, intercostal, and transversus abdominis blood flow increased ($p<0.05$) from rest to moderate-intensity exercise, but no differences ($p>0.05$) existed among groups. *, significantly different compared to rest

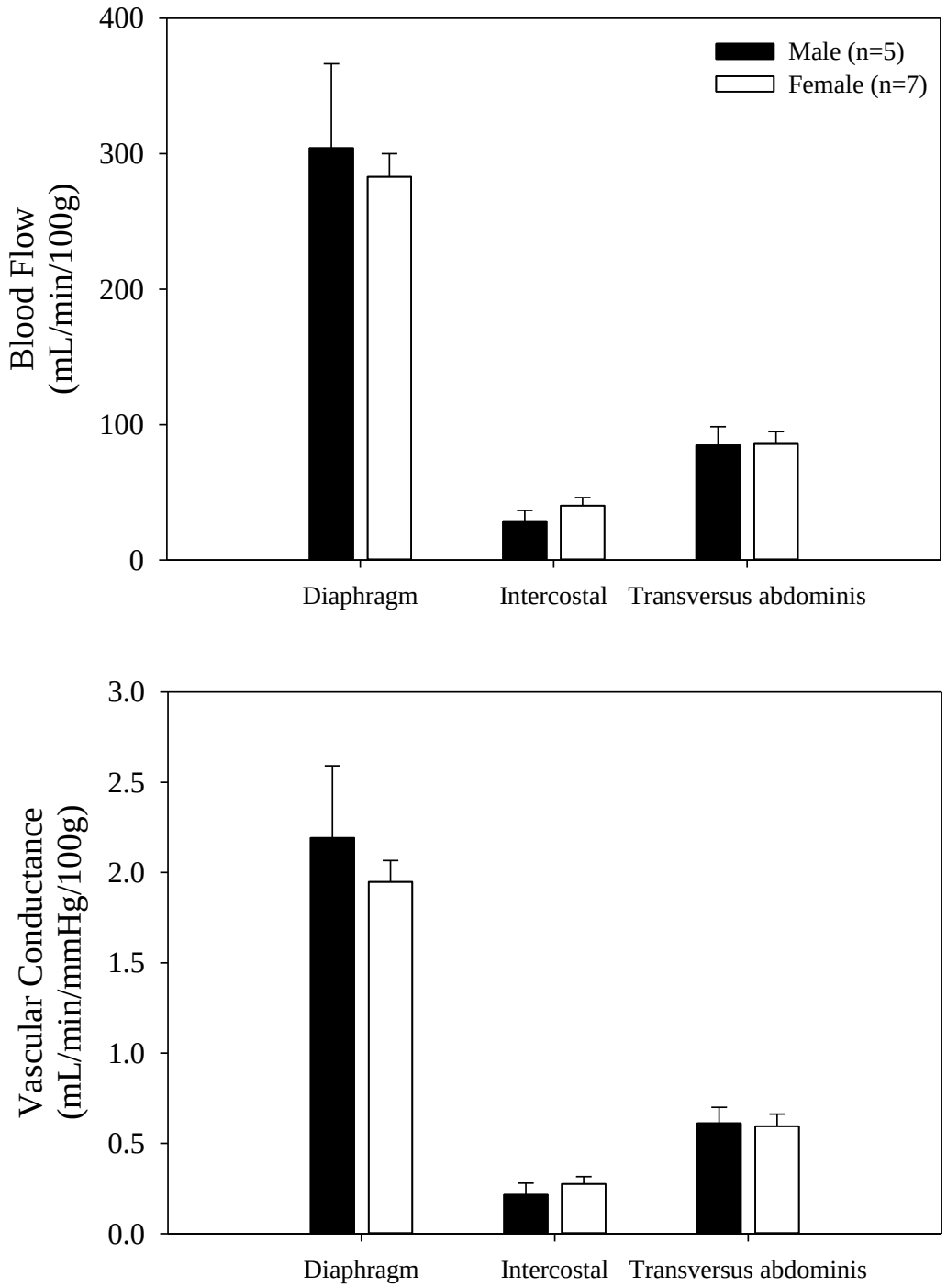

Figure 2-2 Respiratory muscle blood flows and vascular conductances at near-maximal exercise.

Diaphragm, intercostal, and transversus abdominis blood flow (top) and vascular conductance (bottom) during near-maximal exercise in males (black bar) and females (white bar). There were no sex differences ($p>0.05$) in respiratory muscle blood flows or vascular conductances.

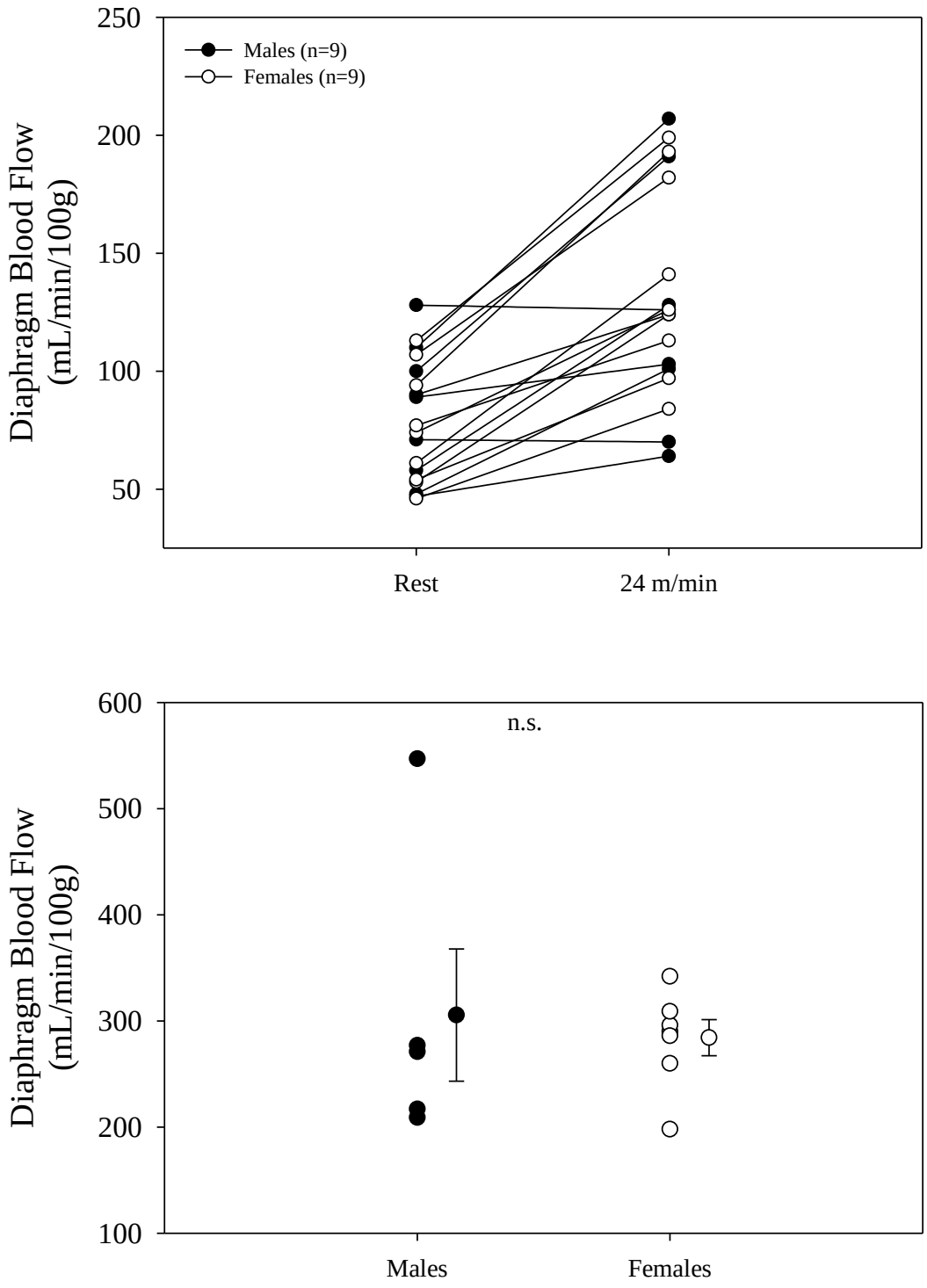

Figure 2-3 Individual diaphragm blood flows at rest and during exercise in males and females.

Male and female individual diaphragm blood flow responses at rest and during moderate-intensity exercise (top) and near-maximal exercise (bottom).

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Chapter 3 - Sex differences in the cardiovascular consequences of the inspiratory muscle metaboreflex

Summary

It is currently unknown if sex differences exist in the cardiovascular consequences of the inspiratory muscle metaboreflex. We hypothesized that the activation of the inspiratory muscle metaboreflex will lead to less of an increase in mean arterial pressure (MAP) and limb vascular resistance (LVR) and less of a decrease in limb blood flow ($\dot{Q}_L$) in women compared to men. Twenty healthy men (n=10, 23 ± 2 yrs) and women (n=10, 22 ± 3 yrs) were recruited for this study. Subjects performed inspiratory resistive breathing tasks (IRBTs) at 2% or 65% of their maximal inspiratory mouth pressure (PI_{MAX}). During the IRBTs, the breathing frequency was 20 breaths min^{-1} with a 50% duty cycle. At rest and during the IRBTs, MAP was measured via automated oscillometry, $\dot{Q}_L$ was measured via Doppler ultrasound, and LVR was calculated. EMG was recorded on the leg to ensure no muscle contraction occurred. The 65% IRBT led to attenuated increases ($p<0.01$) from baseline in women compared to men for MAP (W: 7.3±2.0 mmHg; M: 11.1±5.0 mmHg) and LVR (W: 17.7%±14.0%; M: 47.9±21.0%) as well as less of a decrease ($p<0.01$) in $\dot{Q}_L$ (W: -7.5±9.9%; M: -23.3±10.2%). These sex differences in MAP, $\dot{Q}_L$, and LVR were still present in a subset of subjects matched for PI_{MAX}. The 2% IRBT resulted in no significant changes in MAP, $\dot{Q}_L$, or LVR across time or between men and women. These data indicate pre-menopausal women exhibit an attenuated inspiratory muscle metaboreflex compared to age-matched men.

Introduction

Fatiguing inspiratory muscle work and the concomitant accumulation of metabolites is associated with neural and cardiovascular consequences (7). For example, Hill (17) reported that fatiguing diaphragmatic contractions lead to increased type IV afferent discharge in the anaesthetized rat. In addition, lactic acid infusion into the phrenic circulation leads to increases in mean arterial pressure (MAP) and decreases in limb blood flow ($\dot{Q}_L$) at rest and during exercise in canines (28). In young men, high inspiratory muscle work activates the inspiratory muscle metaboreflex leading to time-dependent increases in muscle sympathetic nerve activity (MSNA), MAP, and leg vascular resistance (LVR), as well as decreases in $\dot{Q}_L$ (29, 37). There is also evidence that the inspiratory muscle metaboreflex is tonically-active during severe-intensity exercise in young men. Harms et al (1997) showed that by unloading the inspiratory muscles (via a proportional assist ventilator) during maximal cycling exercise, LVR decreased ~7% and $\dot{V}O_{2leg}/\dot{V}O_{2tot}$ increased ~10% compared to control (13). Therefore, the inspiratory muscle metaboreflex leads to neural and cardiovascular consequences during severe-intensity exercise.

Sex differences exist in airway size and respiratory mechanics during exercise. For example, women have smaller airways compared to men matched for lung size (31), which is associated with the development of expiratory flow limitation during exercise (12, 36). Consequently, women have a higher work and cost of breathing for a given ventilation (8, 12). Despite the greater work of breathing, women exhibit less diaphragmatic fatigue compared to men (11). Due to the greater work/cost of breathing in women compared to men, it has been suggested that women have an exaggerated inspiratory muscle metaboreflex, consequently leading to greater $\dot{Q}_L$ redistribution away from the exercising locomotor muscles.

Importantly, sex differences also exist in the cardiovascular and neural response to acute stress (38). For example, studies investigating the skeletal muscle metaboreflex (i.e. static handgrip) have found that pre-menopausal women exhibit an attenuated development of metabolites and increase in MSNA and MAP compared to men (9, 21). Furthermore, sex differences exist in peripheral transduction of sympathetic outflow to the peripheral vasculature (14, 18), with women exhibiting less vasoconstriction for a given MSNA compared to men (18). However, it is not known if the activation of the metaboreflex in women leads to less of an increase in LVR and decrease in $\dot{Q}_L$ compared to men. The purpose of this study, therefore, was to determine if sex differences exist in the inspiratory muscle metaboreflex. Based on the sex differences in inspiratory muscle fatigue resistance and metaboreflex-induced increases in metabolites, MSNA, and MAP, we hypothesized that the activation of the inspiratory muscle metaboreflex will lead to (1) attenuated increases in MAP and LVR as well as (2) an attenuated decrease in $\dot{Q}_L$ in pre-menopausal women compared to age-matched men.

Methods

Subjects: Physically-active adults between the ages of 18-35 years were recruited for this project (n=20; 10 men, 10 pre-menopausal women). All subjects completed a detailed medical health history questionnaire prior to entering the study and had normal pulmonary function as assessed by pulmonary function tests (PFTs). Exclusion criteria included smoking history and/or existence of acute and/or chronic cardiovascular, pulmonary, or metabolic diseases. Pre-menopausal women were tested during the follicular phase (days 0-7) of their menstrual cycle as reproductive hormones have been shown to influence cardiovascular function and autonomic control (27). All women were normally menstruating for at least the past 6 months. All subjects refrained from exercise 12 hours and food and caffeine ingestion 2 hours prior to testing. All testing protocols for human subjects were approved by the Kansas State University Institutional Review Board**.**

Experimental design: Subjects visited the laboratory on three different occasions. On the first visit, subjects were familiarized with all procedures and measurements. The next two visits were randomized and subjects performed inspiratory resistive breathing tasks (IRBTs) at 2% or 65% of their maximal inspiratory mouth pressure (PI_{MAX}). The 65% IRBT has previously been shown to induce the inspiratory muscle metaboreflex (29, 30). $\dot{Q}_L$, LVR, MAP, systolic blood pressure (SBP), diastolic blood pressure (DBP) and heart rate (HR) were measured at baseline and during the IRBTs. Surface electromyography (EMG) sensors were placed on the leg to ensure no muscle contractions occurred during the IRBTs. To determine day-to-day variability in the cardiovascular responses during the 65% IRBT, a subset (n=3) of subjects performed three trials of the 65% IRBT on different days at least 24 hours apart.

Inspiratory resistive breathing tasks: First, subjects were seated for >15 min to ensure baseline cardiovascular measurements. Subjects breathed through a custom made inspiratory resistive

breathing device set at 2% or 65% PI_{MAX}. For the custom made inspiratory resistive breathing device, the subjects maximally inhaled through the device and the resistance was increased until it equaled 2% or 65% PI_{MAX}. The custom made inspiratory resistive breathing device consisted of a mouthpiece attached to a step down adapter which allowed for the connection of various diameter tubes used to achieve the desired resistance. Throughout both IRBTs, subjects maintained a breathing frequency of 20 breaths min^{-1} and a prolonged duty cycle (T_I/T_{TOT}= 0.5; 1.5 s inspiration and 1.5 s expiration). Termination of the 65% IRBT was based upon the presence of a plateau in the increase in MAP as previously reported (39). A plateau was defined as ≤2 mmHg absolute increase in MAP over three measurements. MAP was measured every two min throughout the 65% IRBT. Previously, the 65% IRBT has been shown to decrease inspiratory muscle blood flow in canines (3), induce the inspiratory muscle metaboreflex (29, 30, 37, 39), and predict the onset of task failure (inspiratory muscle fatigue) in humans (1, 2, 10, 29, 30). The subjects performed the 2% IRBT for 10 min and it served as the control condition with the same breathing frequency and duty cycle as the 65% IRBT. Expiration was passive during the IRBTs. The investigators visually monitored the subject during the IRBTs to ensure proper timing, breathing technique, and effort. End-tidal carbon dioxide was monitored (SensorMedics 229 Metabolic Cart, SensorMedics Corp., Yorba Linda, CA) each min to determine whether hypocapnia occurred. From pilot work, we expected a slight hypocapnic state to occur during the IRBTs; however, it is unlikely this influenced our results because moderate hypocapnia (~30 mmHg) has been reported to not alter LVR or MAP (15).

Doppler ultrasound: Doppler ultrasound (Vivid 3; GE Medical Systems, Milwaukee WI, USA) was used to measure superficial femoral artery velocity. The gate of the Doppler was set to the full width of the superficial femoral artery to ensure complete insonation. Measurements in the

superficial femoral artery were made approximately 3 cm distal to the bifurcation of the deep and superficial femoral artery. Mean blood velocity (V_{MEAN}; cm x s^{-1}) was defined as the time-averaged mean velocity over each complete cardiac cycle. V_{MEAN} was averaged over the last 15 s of consecutive cardiac cycles for each min. $\dot{Q}_L$ was calculated as the product of vessel cross-sectional area and V_{MEAN}. Two-dimensional sonography was used to measure vessel diameters and calculate cross-sectional area ($CSA=\pi r^2$). Because this ultrasound does not allow for simultaneous measurements of velocity and diameter, the timing of the image capture occurred during diastole and one vessel diameter was used at each time point to calculate blood flow as previously done (34, 35). $\dot{Q}_L$ was calculated at baseline and every min during the IRBTs.

Blood pressure: SBP, DBP and HR were measured at baseline and during the IRBTs via automated blood pressure oscillometry and 3-lead ECG, respectively (S/5 Light Monitor; GE Healthcare; Madison WI). During the IRBTs, HR was recorded each min and SBP and DBP were measured every 2 min. MAP was calculated as 1/3(SBP-DBP) + DBP. LVR was calculated as MAP divided by $\dot{Q}_L$.

Pulmonary function: PFTs were assessed according to American Thoracic Society/European Respiratory Society guidelines. Maximum flow-volume loops were used to establish normal pulmonary function and give measures of forced vital capacity (FVC), forced expiratory volume in 1 s (FEV_1), and peak expiratory flow (PEF) (SensorMedics 229 Metabolic Cart, SensorMedics Corp., Yorba Linda, CA) and these values were expressed as percent predicted (24). PI_{MAX} was used to determine inspiratory muscle strength and was measured from residual volume as previously reported (5, 25).

Statistics: SigmaStat (Janel Scientific Software, Chicago, IL) was used for statistical analysis. Data are expressed as mean±SD. Subject characteristics and resting cardiovascular

variables (MAP, SBP, DBP, $\dot{Q}_L$, LVR, and HR) were compared for sex differences via unpaired *t*-tests. During the IRBTs, MAP, SBP, DBP, HR were reported as an absolute change from baseline and $\dot{Q}_L$ and LVR were reported as a percent change from baseline. For the 2% IRBT, the cardiovascular variables were compared at baseline, min 2, min 4, min 6, min 8, and min 10 via a two-way mixed factorial measures analysis of variance (ANOVA) (sex x time). For the 65% IRBT, the cardiovascular variables were compared at baseline, min 2, min 4, min 6, and the final min via a two-way mixed factorial ANOVA (sex x time). A Tukey's post hoc analysis was performed to determine where significant differences existed. To compare MAP, $\dot{Q}_L$ and LVR between men and women matched for PI_{MAX} (n=5), one-tailed unpaired *t*-tests were performed. Statistical significance was set at $p<0.05$ for all analyses.

Results

Subject characteristics: Table 1 shows subject characteristics. Women were shorter ($p<0.01$) and had a lower body weight ($p=0.01$) compared to men. In addition, women had a lower PI_{MAX} ($p=0.05$), FVC ($p<0.01$), FEV_1 ($p<0.01$), and PEF ($p<0.01$) compared to men. Percent predicted FVC, FEV_1, PEF, and PI_{MAX} were not significantly different (all $p>0.10$) between men and women. SBP ($p<0.01$) and MAP ($p=0.01$) were lower for women, while DBP ($p=0.08$) and HR (W: 68.0±9.6 bpm; M: 69.8±12.2 bpm; $p=0.72$) were not different compared to men. Resting $\dot{Q}_L$ was also lower ($p=0.04$) for women compared to men (W: 73.1±14.2 mL/min; M: 91.9±23.3 mL/min); however, no sex differences existed ($p=0.87$) in QL when scaled to body weight (W: 1.17±0.25 mL/kg/min; M: 1.19±0.34 mL/kg/min). LVR was not significantly different ($p=0.10$) between men and women (W: 1.23±0.23 mmHg/mL/min; M: 1.03±0.27 mmHg/mL/min).

Cardiovascular measures during 2% IRBT: Figure 1 shows the mean absolute change in MAP (1A), SBP (1B), and DBP (1C) from baseline during the 2% IRBT. MAP was not significant as a main effect of time ($F=0.45$; $p=0.81$), sex ($F<0.01$; $p=0.99$), or as an interaction ($F=0.77$; $p=0.58$). For SBP, there was no significant main effect of time ($F=0.46$; $p=0.94$), sex ($F<0.01$; $p=0.80$), or as an interaction ($F=1.49$; $p=0.20$). There was not a significant main effect of time ($F=1.51$; $p=0.20$), sex ($F<0.01$; $p=0.96$), or as an interaction ($F=0.86$; $p=0.51$) for DBP. HR was not significant as a main effect of time ($F=0.36$; $p=0.88$), sex ($F=0.02$; $p=0.90$), or as an interaction ($F=0.79$; $p=0.56$). Figure 2 shows the mean percent changes from baseline in $\dot{Q}_L$ (2A) and LVR (2B). For $\dot{Q}_L$, there was not a significant main effect of time ($F=0.65$; $p=0.66$), sex ($F=0.03$; $p=0.86$), or as an interaction ($F=0.18$; $p=0.97$). There was not a significant main effect of time ($F=0.56$; $p=0.73$), sex ($F=0.02$; $p=0.90$), or as an interaction ($F=0.25$; $p=0.94$) for LVR. End-tidal CO_2 was not different as a main effect of time ($F=0.97$; $p=0.44$), sex ($F=0.74$;

p=0.40), or as an interaction (F=0.38; p=0.86) during the 2% IRBT. During the 2% IRBT, no muscle contraction was measured via EMG.

Cardiovascular measures during 65% IRBT: The mean time for the 65% IRBT was 802±247 s and was not different (p=0.80) between men (816±258 s) and women (787±248 s). Figure 3 shows the mean absolute change during the 65% IRBT and the individual data at the final min for MAP (3A,B), SBP (3C,D), and DBP (3E,F). For MAP, there was a main effect of time (F=14.61; p<0.01) and sex (F=12.71; p<0.01), but not an interaction (F=1.17; p=0.33). Both men (p<0.01) and women (p<0.01) exhibited an absolute increase in MAP from baseline to the final min. Women showed less of an absolute increase in MAP at min 2 (p=0.02), min 6 (p=0.01), and the final min (p=0.05) compared to men. For SBP, there was a main effect of time (F=10.84; p<0.01), but not sex (F=2.16; p=0.16) or interaction (F=0.29; p=0.88). SBP increased for men (p<0.01) and women (p<0.01) from baseline to the final min. For DBP, there was a main effect of time (F=9.84; p<0.01) and sex (F=11.25; p<0.01), but not an interaction (F=1.44; p=0.23). The absolute DBP increased from baseline to the final min for men (p<0.01), but not women (p=0.16). Women exhibited less of an absolute increase in DBP at min 2 (p=0.04), min 6 (p<0.01), and the final min (p=0.04) during the 65% IRBT compared to men. There was a significant main effect of time (F=12.22; p<0.01), sex (F=7.17; p=0.02), and an interaction (F=3.48; p=0.01) for HR. HR significantly increased from baseline to the final min in men (p<0.01), but not women (p=0.10). Furthermore, women had less of an increase in HR at min 2 (p=0.05), min 4 (p<0.01), min 6 (p=0.05) and the final min (p<0.01) compared to men. At the final min, the absolute change in HR in men was 9.7±3.1 bpm and in women was 4.0±3.7 bpm.

Figure 4 shows the mean percent change during the 65% IRBT and the individual data at the final min for $\dot{Q}_L$ (4A,B) and LVR (4C,D). There was a main effect of time (F=9.47; p<0.01),

sex (F=13.21; p<0.01), and interaction (F=4.99; p<0.01) for the percent change of $\dot{Q}_L$. There was a significant decrease in $\dot{Q}_L$ from baseline to the final min for men (p<0.01), but not women (p=0.53). Women had less of a decrease in $\dot{Q}_L$ at min 2 (p<0.01), 6 (p<0.01) and the final min (p=0.01) of the 65% IRBT compared to men. For LVR, there was a main effect of time (F=12.88; p<0.01), sex (F=9.69; p=0.01), and interaction (F=5.51; p<0.01). There was a significant increase in LVR from baseline to the final min of the 65% IRBT in men (p<0.01), but not women (p=0.27). Women showed less of an increase in LVR at min 2 (p<0.01), min 6 (p<0.01), and the final min (p=0.01) of the 65% IRBT. For the superficial femoral diameter, there was a main effect of time (F=3.69; p=0.01), but not sex (F=1.77; p=0.20) or as an interaction (F=1.98; p=0.11). For men, superficial femoral diameter at the final min was significantly smaller (p=0.03) compared to baseline. Final min superficial femoral diameter was not different (p=0.93) compared to baseline for women. No relationships (p>0.05) existed between PI_{MAX} and the changes in MAP, $\dot{Q}_L$ or LVR at the final min of the 65% IRBT for all subjects, men or women. There was no main effect of time (F=2.16; p=0.08), sex (F=0.24; p=0.63), or interaction (F=0.41; p=0.80) for end-tidal CO_2 during the 65% IRBT. During the 65% IRBT, no muscle contraction was measured via EMG. Across the three trials of the 65% IRBT performed by the subset of subjects (n=3; 2M/1W), the mean coefficient of variation across time (i.e. min 2, min 4, min 6, and the final min) for MAP was 3.5%, $\dot{Q}_L$ was 13%, and LVR was 14%.

Sex comparisons when matched for PI_{MAX}: Figure 5 shows the mean absolute change in MAP and mean percent change in $\dot{Q}_L$ and LVR at the final min of the 65% IRBT in a subset of men (n=5) and women (n=5) matched for PI_{MAX}. PI_{MAX} (5A) was not different (p=0.67) between men and women in this subset. At the final min, women had less of an absolute increase in MAP

(5B) (p=0.03) and percent change increase in LVR (5D) (p=0.01) from baseline compared to men. In addition at the final min, women exhibited less of a percent decrease in $\dot{Q}_L$ (5C) (p=0.01) from baseline. These results were similar to those of the entire groups, as well.

Discussion

Major findings: The major findings of the current study are that with the activation of the inspiratory muscle metaboreflex pre-menopausal women exhibit less of a(n) 1) increase in blood pressure (MAP and DBP) and LVR as well as 2) decrease in $\dot{Q}_L$ compared to men. Furthermore, these sex differences in MAP, $\dot{Q}_L$, and LVR were still present when PI_{MAX} was matched. These findings suggest pre-menopausal women have an attenuated inspiratory muscle metaboreflex compared to age-matched men.

Inspiratory muscle metaboreflex: Increased inspiratory muscle work leads to time-dependent neural and cardiovascular consequences. Specifically, high inspiratory muscle work (above 60%MIP (30)) leads to time dependent increases in MSNA leading to increases in MAP and LVR as well as decreases in $\dot{Q}_L$ (29, 30, 37). The magnitudes of change of MAP, $\dot{Q}_L$, and LVR in men in the present study are in excellent agreement with these previous studies. We and others (4, 29, 37) have shown that maintaining the same breathing frequency and duty cycle without the high inspiratory muscle load does not lead to changes in MAP, $\dot{Q}_L$, LVR or MSNA suggesting the high inspiratory muscle work-induced metabolic accumulation is responsible for these cardiovascular and neural responses. In support of this, fatiguing diaphragmatic contractions in the anesthetized rat leads to increased type IV (metabosensitive) afferent discharge (17). Furthermore, stimulation of the phrenic afferents leads to vasoconstriction and decreases in blood flow (19). Other sources such as central command and mechanoreflex may also contribute to the neural and cardiovascular responses observed in the current study. Regarding central command, it has previously been shown that performing the IRBT at near maximal inspiratory pressures (without inducing fatigue) does not elicit increases in MSNA, MAP, LVR, or decreases in $\dot{Q}_L$ until after three min (29, 37). In addition, the 2% IRBT did not

lead to increases in MAP suggesting the mechanoreflex was not activated. However, metabolite accumulation has been shown to increase type III (mechanosensitive) afferent activity (33) suggesting that it is possible the mechanoreflex may have contributed to the MAP response during the 65% IRBT.

Sex differences in the inspiratory muscle metaboreflex: Cardiovascular and neural sex differences have previously been reported (22). For example, women generally have a lower resting blood pressure and MSNA compared to men (22). In regards to the current study, pre-menopausal women have been reported to have an attenuated skeletal muscle metaboreflex compared to age-matched men (9, 21). Specifically, women have less metabolite accumulation (H^+ and $H_2PO_4^-$) as well as an attenuated increase in MSNA and MAP during static handgrip exercise and post-exercise circulatory occlusion (9, 21). In contrast to these studies, it has been speculated that women may have a greater inspiratory muscle metaboreflex because of their greater work and cost of breathing for a given ventilation (8, 12). In the present study, we found that women exhibited an attenuated MAP response compared to men consistent with previous findings (9, 21). Furthermore, we found that the activation of the inspiratory muscle metaboreflex in women led to an attenuated rise in LVR and decrease in $\dot{Q}_L$ compared to men. Importantly, these sex differences were still present when maximal inspiratory pressure was matched, consistent with previous investigations of the skeletal muscle metaboreflex (9).

What are potential mechanisms for this attenuated inspiratory muscle metaboreflex in women? Possibilities include sex differences in substrate utilization, muscle morphology, and vascular transduction. To date, sex differences in inspiratory muscle fiber types have not been investigated; however, it has previously been shown that women have a greater percentage of type I muscle fibers compared to men in other skeletal muscles (32). Although the diaphragm is

composed of mainly oxidative muscle fibers (76% highly oxidative fibers and 24% low oxidative fast-twitch fibers (26)), it is possible that women exhibit a greater percentage of type I/IIa muscle fibers in the diaphragm. In addition, women have been suggested to have a greater reliance on β-oxidation of fatty acids (16). Both of these mechanisms would contribute to the attenuated metabolite production as well as the greater inspiratory muscle fatigue resistance previously reported in young women compared to young men (10, 11). In addition, sex differences exist in the peripheral transduction of sympathetic outflow to the peripheral vasculature (14, 18). Specifically, the same degree of MSNA leads to greater limb vasoconstriction in men compared to women (18). The sex differences in the transduction of MSNA to the peripheral vasculature are thought to be influenced by blunted α-adrenergic vasoconstriction due to greater β-adrenergic vasodilation in pre-menopausal women (14). It is possible the arterial baroreceptors also contributed to the attenuated blood pressure response in women. The arterial baroreceptors have been shown to interact with the skeletal muscle metaboreflex for control of neural and cardiovascular changes (6, 20). Previously, the baroreflex-mediated depressor response to carotid hypertension was shown to be greater in women compared to men suggesting the arterial baroreceptors may have contributed to the attenuated MAP response (23). It is currently not known if sex differences are present in the baroreflex sensitivity during metaboreflex activation.

Limitations: Several potential limitations may have influenced our results. First diaphragmatic fatigue was not directly assessed in the current study. However, this protocol (i.e. 65%PI_{MAX}, breathing frequency, duty cycle) has previously been shown to elicit inspiratory muscle fatigue in men (10, 29, 30, 37) and women (10) thus inspiratory muscle fatigue likely occurred in the current study during the 65% IRBT. Future studies are needed to further evaluate the degree of diaphragmatic fatigue in women following IRBTs. Second, inspiratory mouth pressure was not

directly measured during the IRBTs. However, the magnitudes of change of MAP, $\dot{Q}_L$, and LVR in the men of the current study are in excellent agreement with previous studies (4, 29, 30, 37) suggesting the protocol was sufficient to activate the inspiratory muscle metaboreflex.

Conclusion: In the present study, it was found that women exhibit a blunted inspiratory muscle metaboreflex compared to age-matched men. In addition, the attenuated cardiovascular consequences were still present when maximal inspiratory pressures were matched. Future studies should examine if sex differences in the inspiratory muscle metaboreflex occur during severe-intensity exercise and if sex differences exist in baroreflex sensitivity with metaboreflex activation.

Table 3-1 Subject Characteristics

	Men	Women	p value
n	10	10	
Age (yrs)	23 ± 2	22 ± 3	p=0.71
Ht (cm)	179 ± 7	163 ± 5*	p<0.01
Wt (kg)	77.6 ± 8.3	63.7 ± 12.3*	p=0.01
SBP (mmHg)	124 ± 6	112 ± 8*	p<0.01
DBP (mmHg)	76 ± 6	71 ± 6	p=0.08
MAP (mmHg)	92 ± 5	85 ± 6*	p=0.01
FVC (L)	5.8 ± 0.5	4.0 ± 0.5*	p<0.01
FVC (% predicted)	108 ± 8	109 ± 9	p=0.82
FEV_1 (L)	4.7 ± 0.5	3.3 ± 0.3*	p<0.01
FEV_1 (%predicted)	101 ± 10	103 ± 7	p=0.65
PEF ($L\ s^{-1}$)	10.6 ± 2.6	6.5 ± 0.9*	p<0.01
PEF (%predicted)	110 ± 29	95.0 ± 13	p=0.15
PI_{MAX} (cmH_20)	178 ± 51	137 ± 40*	p=0.05
PI_{MAX} (%predicted)	142 ± 43	151 ± 41	p=0.62

Ht, height; Wt, weight; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; FVC, forced vital capacity; FEV_1, forced expiratory volume in 1 s; PEF, peak expiratory flow; PI_{MAX}, maximal inspiratory mouth pressure; *, p<0.05

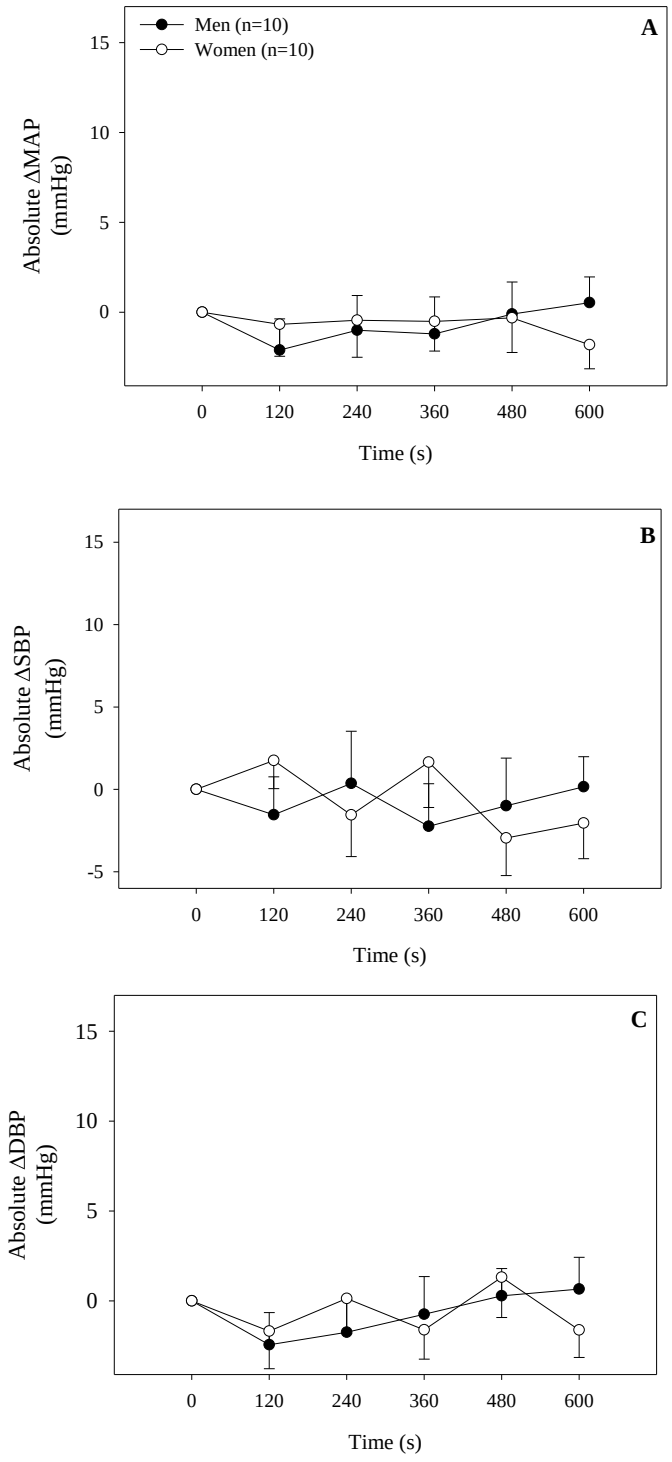

Figure 3-1 Blood pressure responses during the 2% IRBT.

The mean absolute change from baseline for MAP (A), SBP (B), and DBP (C) for men (closed circles) and women (open circles) during the 2% IRBT. There were no significant main effects of time (all $p>0.20$) or sex ($p>0.90$) for any of the blood pressure responses.

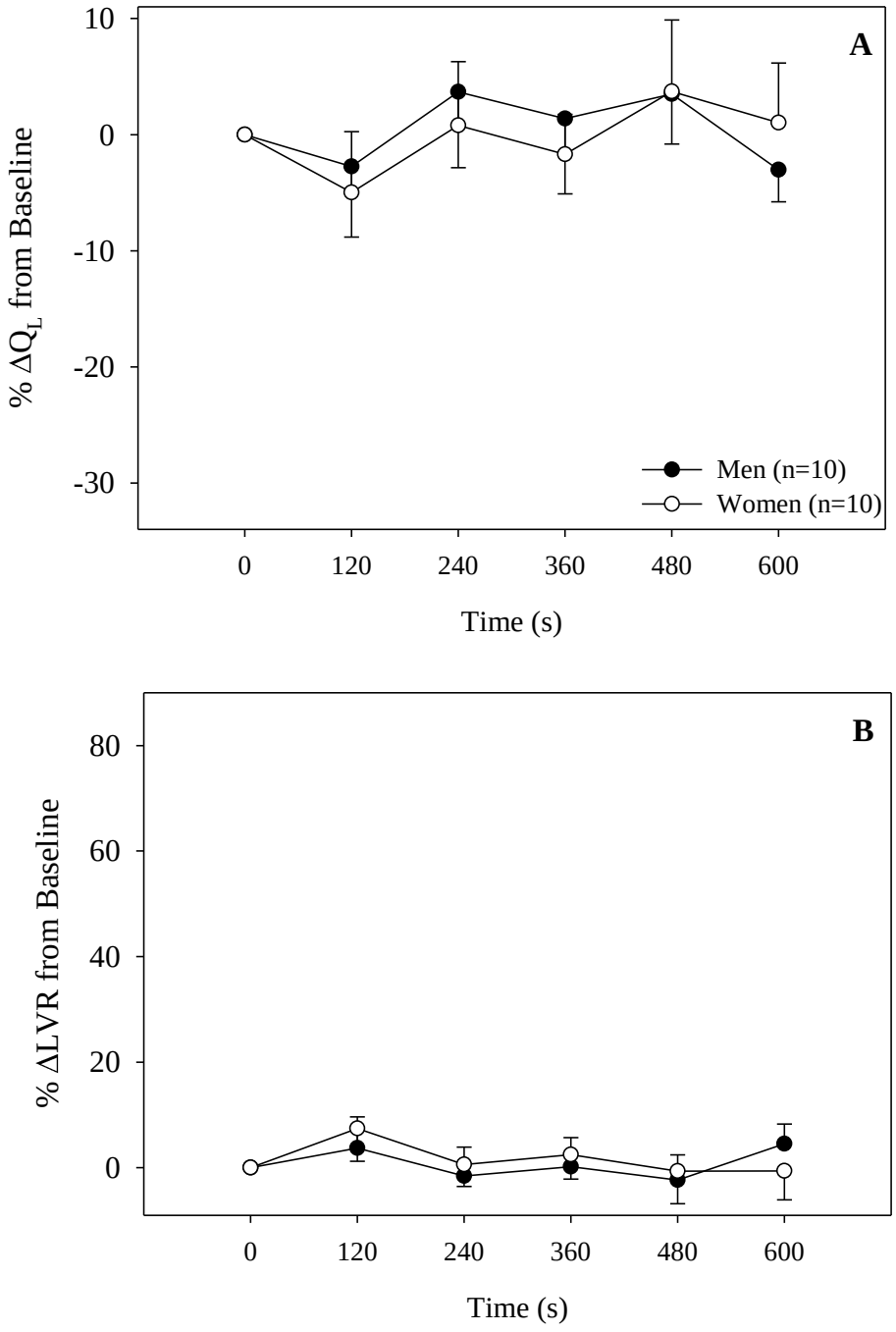

Figure 3-2 $\dot{Q}_L$ and LVR responses during the 2% IRBT.

The mean percent change from baseline for $\dot{Q}_L$ (A) and LVR (B) for men (closed circles) and women (open circles) during the 2% IRBT. There were no significant main effects of time (all $p>0.60$) or sex (all $p>0.80$) for $\dot{Q}_L$ or LVR during the 2% IRBT.

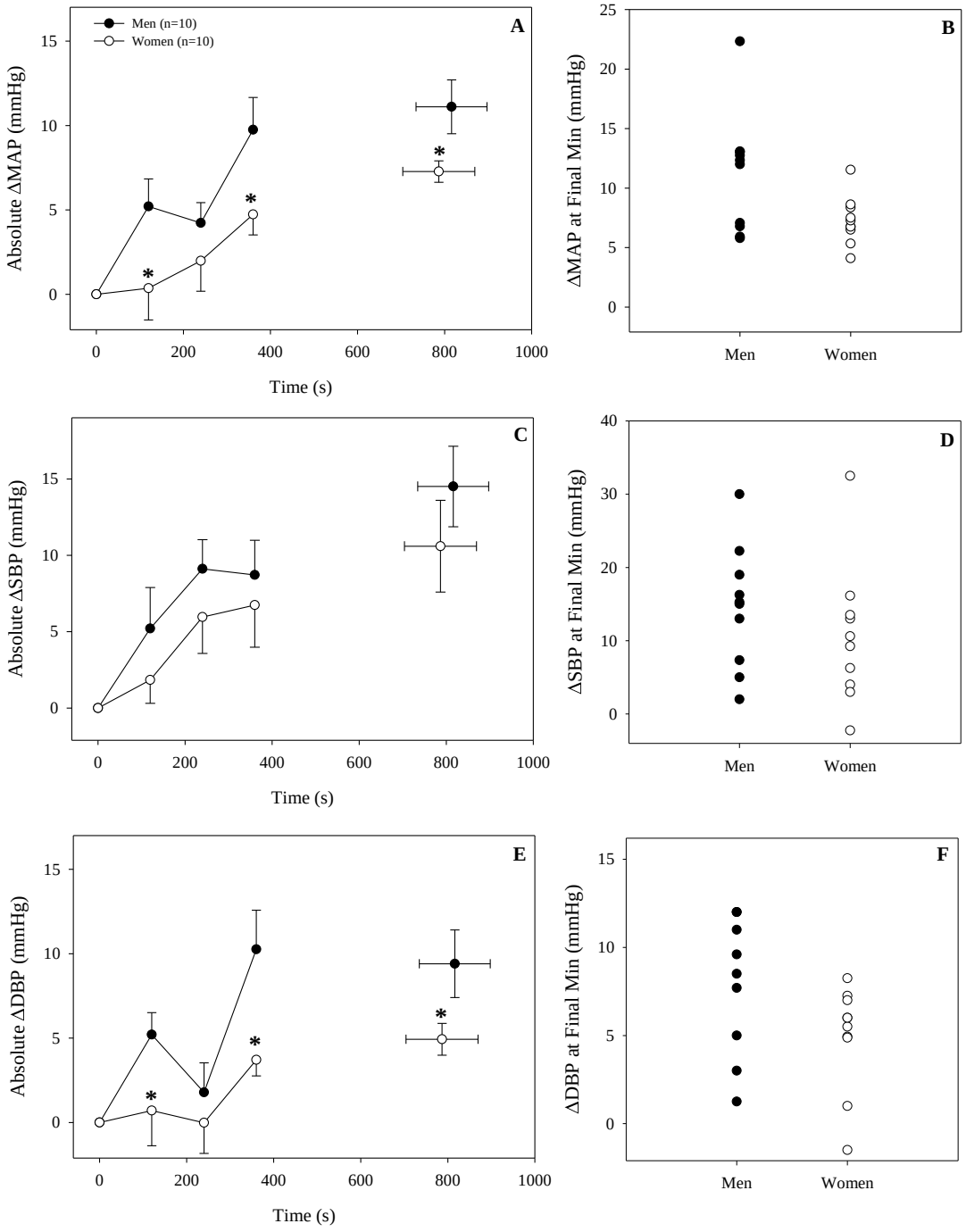

Figure 3-3 Blood pressure responses during the 65% IRBT.

The mean absolute change from baseline and individual data at the final min for MAP (A,B), SBP (C,D), and DBP (E,F) for men (closed circles) and women (open circles) during the 65% IRBT. There were significant ($p<0.05$) main effects of time for MAP, SBP and DBP and for sex for MAP and DBP. * significantly different from men.

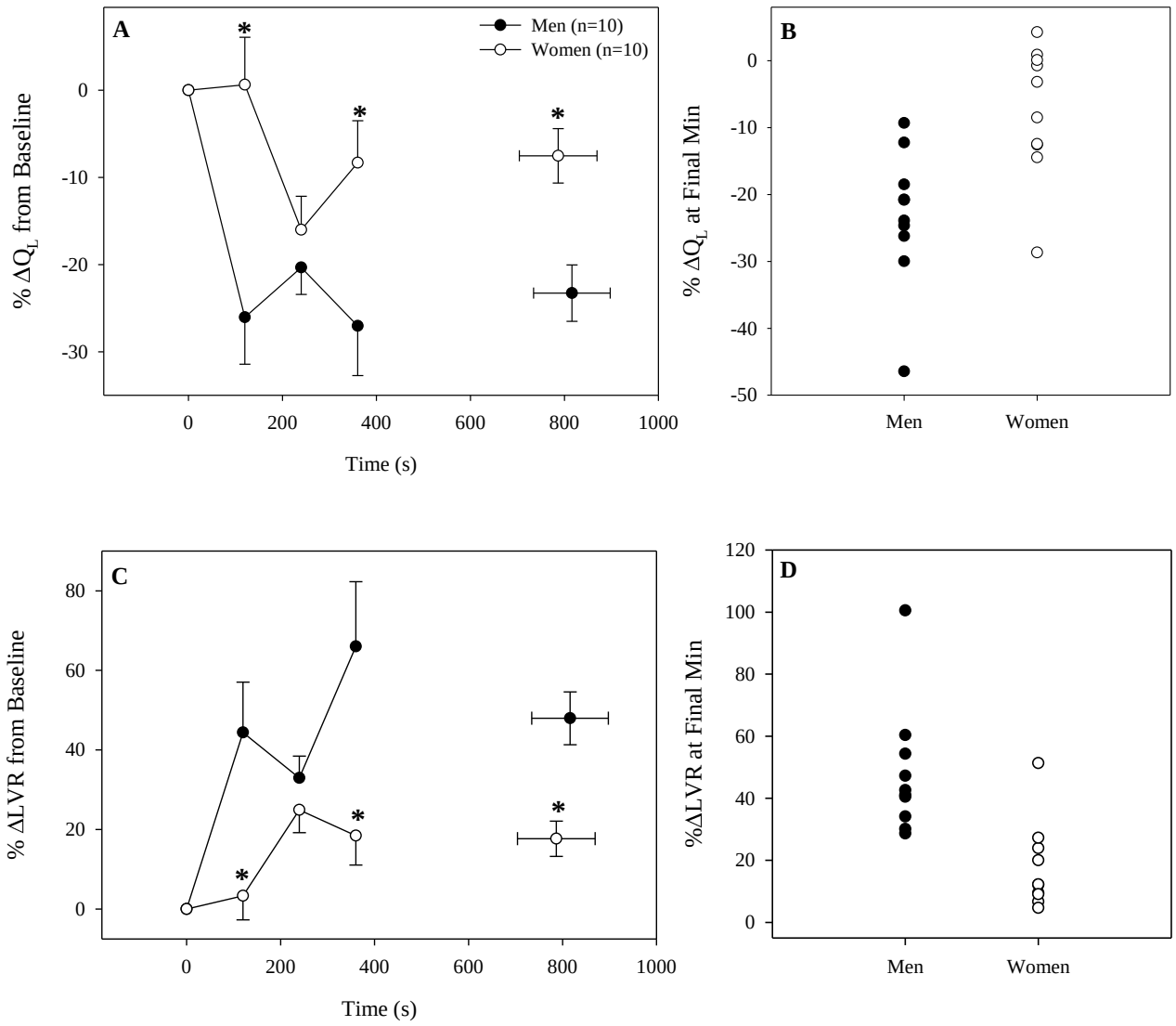

Figure 3-4 $\dot{Q}_L$ and LVR responses during the 65% IRBT.

The mean percent change from baseline and individual data at the final min for $\dot{Q}_L$ (A, C) and LVR (B, D) for men (closed circles) and women (open circles) during the 65% IRBT. There were significant ($p<0.05$) main effects of time and sex for QL and LVR. * significantly different from men.

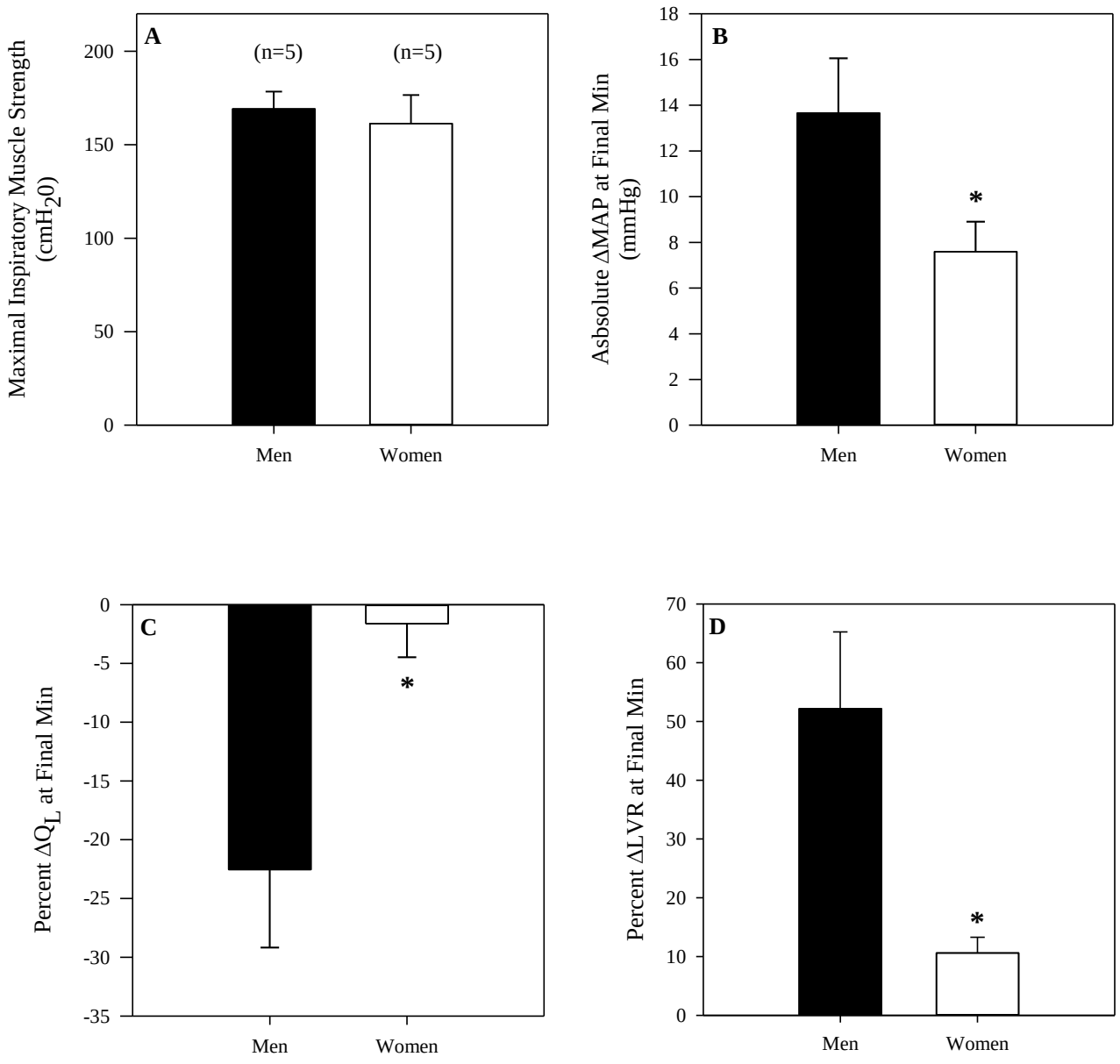

Figure 3-5 Cardiovascular comparisons when matched for PI_{MAX}.

Sex differences in MAP, $\dot{Q}_L$, and LVR when matched for PI_{MAX}. Men had greater increases in MAP ($p=0.03$) and LVR ($p=0.01$) and decreases in $\dot{Q}_L$ ($p=0.01$) compared to women when PI_{MAX} were not different ($p=0.67$).

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Chapter 4 - Cardiovascular consequences of the inspiratory muscle metaboreflex: effects of age and sex

Summary

We hypothesized that, compared to their younger counterparts, older men and women would exhibit greater 1) increases in mean arterial pressure (MAP) and limb vascular resistance (LVR) and 2) decreases in limb blood flow ($\dot{Q}_L$), but 3) no sex differences would be present in older adults. Sixteen young (8 men (YM), 8 women (YW); 18-24 yrs) and older (8 men (OM), 8 women (OW); 60-73 yrs) adults performed inspiratory resistive breathing tasks (IRBTs) at 2% and 65% of their maximal inspiratory pressure. During the IRBTs, breathing frequency was 20 breaths min^{-1} with a 50% duty cycle. At baseline and during the IRBTs, MAP was measured via automated oscillometry, $\dot{Q}_L$ was determined via Doppler ultrasound, and LVR was calculated. The 65% IRBT led to significantly greater increases in MAP in OW (15.9±8.1mmHg) compared to YW (6.9±1.4mmHg), but not ($p>0.05$) between OM (12.3±5.7mmHg) and YM (10.8±5.7 mmHg). OW (-20.2±7.2%) had greater ($p<0.05$) decreases in $\dot{Q}_L$ compared to YW (-9.4±10.2%), but no significant differences were present between OM (-22.8±9.7%) and YM (-22.7±11.3%) during the 65% IRBT. The 65% IRBT led to greater ($p<0.05$) increases in LVR in OW (48.2±25.5%) compared to YW (19.7±15.0%), but no differences ($p>0.05$) existed among OM (54.4±17.8%) and YM (47.1±23.3%). No significant differences were present in MAP, $\dot{Q}_L$, or LVR between OM and OW. These data suggest OW exhibit a greater inspiratory muscle metaboreflex compared to YW, while no differences between OM and YM existed. Lastly, sex differences in the inspiratory muscle metaboreflex are not present in older adults.

Introduction

Fatiguing inspiratory muscle work and the concomitant accumulation of metabolites is associated with neural and cardiovascular consequences (10). Specifically, fatiguing diaphragmatic contractions lead to increased type IV afferent discharge in the anaesthetized rat (21). In addition, lactic acid infusion into the phrenic circulation leads to increases in mean arterial pressure (MAP) and decreases in limb blood flow ($\dot{Q}_L$) at rest and during exercise in canines (40). In young men, high inspiratory muscle work activates the inspiratory muscle metaboreflex, leading to time-dependent increases in muscle sympathetic nerve activity (MSNA), MAP, and leg vascular resistance (LVR), as well as decreases in $\dot{Q}_L$ (42, 49). There is also evidence that the inspiratory muscle metaboreflex is tonically-active during severe-intensity exercise in young men. Harms et al (1997) showed that by unloading the inspiratory muscles (via a proportional assist ventilator) during maximal cycling exercise, LVR decreased ~7% and $\dot{V}O_{2leg}/\dot{V}O_{2tot}$ increased ~10% compared to control (18). Therefore, the inspiratory muscle metaboreflex leads to neural and cardiovascular consequences during severe-intensity exercise.

Aging leads to changes within the pulmonary system such as decreases in respiratory muscle strength (17) and expiratory flow rates (28, 48), loss of elastic recoil (28), and stiffening of the chest wall (26). Consequently, dynamic compliance is reduced with aging, leading to a higher cost and work of breathing for a given ventilation compared to younger individuals (26). Furthermore, older adults have been reported to have less inspiratory muscle fatigue resistance compared to younger adults (5). Based on these aging-induced changes to the pulmonary system, it is likely that older adults will exhibit an exaggerated inspiratory muscle metaboreflex compared to younger adults.

We have recently observed sex differences in the inspiratory muscle metaboreflex (47); however, the influence of age on the inspiratory muscle metaboreflex was not evaluated in our previous study, and is unknown. Therefore, the purpose of this study was to determine the effect of age on sex differences in the cardiovascular consequences of the inspiratory muscle metaboreflex. We hypothesized that compared to their younger counterparts the activation of the inspiratory muscle metaboreflex in older men and women would lead to greater increases in MAP and LVR resulting in decreases in $\dot{Q}_L$. In addition, we hypothesized that the inspiratory muscle metaboreflex-induced cardiovascular responses would not be different between older men and women.

Methods

Subjects: Young adults (n=16; 8 men (YM), 8 pre-menopausal women (YW)) and older adults (n=16; 8 men (OM), 8 post-menopausal women (OW)) were recruited for this project. The young adults were between the ages of 18-35 years and the older adults were ≥60 years of age. All subjects completed a detailed medical health history questionnaire and signed an informed consent prior to entering the study and had normal pulmonary function as assessed by pulmonary function tests (PFTs). Exclusion criteria included existence of acute and/or chronic cardiovascular, pulmonary, or metabolic diseases. Endurance-trained subjects were not recruited in the present study because endurance-training results in increased inspiratory muscle fatigue resistance (33) and attenuated inspiratory muscle metaboreflex (4, 25). All pre-menopausal women were normally menstruating for at least the past 6 months and were tested during the early follicular phase (days 0-7) of their menstrual cycle as reproductive hormones have been shown to influence cardiovascular function and autonomic control (35). All older women were post-menopausal. All subjects refrained from exercise for 12 hours and food and caffeine ingestion for 2 hours prior to testing. All testing protocols for human subjects were approved by the Kansas State University Institutional Review Board and conformed to the principles in the Declaration of Helsinki.

Experimental design: Subjects visited the laboratory on three different occasions. On the first visit, subjects were familiarized with all procedures and measurements. The next two visits were randomized and subjects performed inspiratory resistive breathing tasks (IRBTs) at 2% or 65% of their maximal inspiratory mouth pressure (PI_{MAX}). The 65% IRBT has previously been shown to induce the inspiratory muscle metaboreflex (42, 43, 47). At baseline and during the IRBTs,

$\dot{Q}_L$, LVR, MAP, and heart rate (HR) were measured. Surface electromyography (EMG) sensors were placed on the leg to confirm that no muscle contractions occurred during the IRBTs.

Inspiratory resistive breathing tasks: First, subjects were seated for >15 min to ensure baseline cardiovascular measurements. Subjects breathed through a custom made inspiratory resistive breathing device set at 2% or 65%PI_{MAX}. For the custom made inspiratory resistive breathing device, the subjects maximally inhaled through the device and the resistance was increased until it equaled 2% or 65%PI_{MAX} as previously done (47). The custom made inspiratory resistive breathing device consisted of a mouthpiece attached to a step down adapter which allowed for the connection of various diameter tubes used to achieve the desired resistance. Throughout both IRBTs, subjects maintained a breathing frequency of 20 breaths min^{-1} and a prolonged duty cycle (T_I/T_{TOT}= 0.5; 1.5 s inspiration and 1.5 s expiration). MAP was measured every two min throughout the 65% IRBT. Termination of the 65% IRBT was based upon the presence of a plateau in the increase in MAP (47, 51), which was defined as ≤2 mmHg absolute increase in MAP over three measurements (47). Termination of the 65% IRBT has previously been shown to occur following 8-20 min (47). The data collected during the third MAP measurement to confirm the plateau in MAP was reported as the "final min" in the Results. Previously, the 65% IRBT has been shown to decrease inspiratory muscle blood flow in canines (3), induce the inspiratory muscle metaboreflex (42, 43, 47, 49, 51), and predict the onset of task failure (inspiratory muscle fatigue) in humans (1, 2, 42, 43). The subjects performed the 2% IRBT for 10 min and it served as the control condition with the same breathing frequency and duty cycle as the 65% IRBT. Expiration was passive during all IRBTs. The investigators visually monitored each breath of the subject to ensure proper timing and effort. End-tidal carbon dioxide was monitored (SensorMedics 229 Metabolic Cart, SensorMedics Corp., Yorba Linda,

CA) each min to ensure subjects were isocapnic during the IRBTs. We have recently shown that the mean coefficient of variation across time for MAP was 3.5%, $\dot{Q}_L$ was 13%, and LVR was 14% during the 65% IRBT (47).

Doppler ultrasound: Doppler ultrasound (Vivid 3; GE Medical Systems, Milwaukee WI, USA) was used to measure superficial femoral artery blood velocity. The gate of the Doppler was set to the full width of the artery to ensure complete insonation. Measurements were made approximately 3 cm distal to the bifurcation of the deep and superficial femoral artery. Mean blood velocity (V_{MEAN}; cm x s^{-1}) was defined as the time-averaged mean velocity over each complete cardiac cycle. V_{MEAN} was averaged over the last 15 s of consecutive complete cardiac cycles for each min. $\dot{Q}_L$ was calculated as the product of vessel cross-sectional area and V_{MEAN}. Two-dimensional sonography was used to measure vessel diameters and calculate cross-sectional area ($CSA=\pi r^2$). Because this ultrasound does not allow for simultaneous measurements of velocity and diameter, the timing of the image capture occurred during diastole and one vessel diameter was used at each time point to calculate blood flow as previously done (45, 46). $\dot{Q}_L$ was calculated at baseline and every min during the IRBTs.

Blood pressure: MAP and HR were measured at baseline and during the IRBTs via automated blood pressure oscillometry and 3-lead ECG, respectively (S/5 Light Monitor; GE Healthcare; Madison WI). During the IRBTs, HR was recorded each min and systolic (SBP) and diastolic blood pressure (DBP) were measured every 2 min. MAP was calculated as 1/3(SBP-DBP) + DBP. LVR was calculated as MAP divided by $\dot{Q}_L$. Rate pressure product (RPP) was determined as the product of SBP and HR. Pulse pressure was determined by subtracting DBP from SBP.

Pulmonary function: PFTs were assessed according to American Thoracic Society/European Respiratory Society guidelines. Maximum flow-volume loops were used to establish normal

pulmonary function and give measures of forced vital capacity (FVC), forced expiratory volume in 1 s (FEV_1), and peak expiratory flow (PEF) (SensorMedics 229 Metabolic Cart, SensorMedics Corp., Yorba Linda, CA) and these values were expressed as percent predicted (29). PI_{MAX} was used to determine inspiratory muscle strength and was measured at residual volume as previously done (6, 31, 33).

Statistics: SigmaStat (Janel Scientific Software, Chicago, IL) was used for statistical analysis. Data are expressed as mean±SD. Subject characteristics and baseline cardiovascular variables (MAP, $\dot{Q}_L$, LVR, HR, RPP, and pulse pressure) were compared between YM and OM, YW and OW, as well as OM and OW via unpaired one-way analysis of variance (ANOVA). During the IRBTs, MAP, HR, RPP, and pulse pressure were reported as an absolute change from baseline and $\dot{Q}_L$ and LVR were reported as a percent change from baseline. For the 2% IRBT, the cardiovascular variables were compared within (baseline, min 2, min 4, min 6, min 8, and min 10) and among (YM, OM, YW, OW) groups via a two-way mixed factorial ANOVA (group x time). For the 65% IRBT, the cardiovascular variables were compared within (baseline, min 2, min 4, and the final min) and among (YM, OM, YW, OW) groups via a two-way mixed factorial ANOVA (group x time). A Student-Newman-Keuls post hoc analysis was performed to determine where significant differences existed. To compare MAP, $\dot{Q}_L$, and LVR between YM and OM matched for PI_{MAX} (n=5), unpaired *t*-tests were performed. Statistical significance was set at $p<0.05$ for all analyses.

Results

Subject characteristics: Table 1 shows subject characteristics for YM, YW, OM, and OW. OM and OW were older ($p<0.01$) than YM and YW, respectively. There were no differences ($p=0.12$) in baseline blood pressure, pulse pressure, or RPP between OM and YM, OW and YW, or OM and OW. OM had lower FVC ($p<0.01$), FEV_1 ($p<0.01$), and PI_{MAX} ($p=0.03$) compared to YM. FEV_1 ($p<0.01$) was lower for OW compared to YW. OM had higher FVC ($p<0.01$), FEV_1 ($p<0.01$), and PEF ($p<0.01$) than OW. % predicted pulmonary function were not different ($p>0.19$) among groups. No differences ($p>0.49$) were present among groups in baseline $\dot{Q}_L$ (YM: 92.2±26.4 mL/min; OM: 89.5±27.4 mL/min; OW: 81.7±28.9 mL/min; YW: 69.1±11.4 mL/min) or LVR (YM: 1.02±0.30 mmHg/mL/min; OM: 1.20±0.40 mmHg/mL/min; OW: 1.23±0.40 mmHg/mL/min; YW: 1.27±0.22 mmHg/mL/min).

Cardiovascular measures during 2% IRBT: Figure 1 shows the cardiovascular responses during the 2% IRBT. The change in MAP and HR were not different among groups ($p>0.23$) or across time ($p>0.27$). Also, the percent change in $\dot{Q}_L$ and LVR were not different among groups ($p>0.81$) or across time ($p>0.80$). End-tidal CO_2 was significantly lower ($p=0.03$) during the 2% IRBT for OW, but not for any other group ($p>0.30$). During the 2% IRBT, no muscle contraction was measured via EMG.

Cardiovascular measures during 65% IRBT: The mean time for the 65% IRBT was not different ($p=0.73$) among YM (12.3±1.3 min), OM (11.4±1.3 min), YW (13.1±1.4 min), and OW (11.5±0.6 min). Figure 2 shows the change in MAP (A) and HR (B) from baseline during the 65% IRBT. During the 65% IRBT, MAP increased ($p<0.02$) from baseline to the final min in all groups. At min 2, the increase in MAP was greater ($p<0.01$) in OW compared to YW. At min 4, the increase in MAP was greater in both OW ($p<0.01$) and OM ($p<0.01$) compared to YW and

YM, respectively. In addition, the increase in MAP was greater ($p=0.04$) in OW compared to OM at min 4. At the final min, MAP was significantly higher ($p<0.01$) for OW compared to YW, while no other differences (all $p>0.15$) were present. HR increased from baseline to the final min for YM ($p<0.01$) and OM ($p<0.01$), but not YW ($p=0.40$) and OW ($p=0.11$). The HR response during the 65% IRBT was not different among groups at min 2 (all $p>0.13$) or 4 (all $p>0.10$). At the final min, OM ($p=0.02$) had a greater increase in HR compared to OW.

The changes in pulse pressure (A) and RPP (B) during the 65% IRBT are shown in Figure 3. Pulse pressure increased from baseline to the final min in OM ($p=0.01$) and OW ($p<0.01$), but not YM ($p=0.37$) or YW ($p=0.61$). At min 2, OM ($p=0.02$) and OW ($p<0.01$) had greater increases in pulse pressure compared to YM and YW, respectively. At min 4, OW had greater increases in pulse pressure compared to YW ($p=0.03$) and OM ($p=0.03$). At the final min, OW had greater increases ($p<0.01$) in pulse pressure than YW. From baseline to the final min, RPP increased in YM ($p<0.01$), OM ($p<0.01$), and OW ($p<0.01$), but not YW ($p=0.57$). The increase in RPP was greater for OW ($p<0.01$) compared to YW at min 2, 4, and the final min.

Figure 4 shows the mean percent change during the 65% IRBT for $\dot{Q}_L$ (A) and LVR (B). From baseline to the final min, $\dot{Q}_L$ decreased ($p<0.03$) in all groups. At min 2, YW had a small non-significant ($p=0.25$) increase in $\dot{Q}_L$ compared to rest, while OW had greater ($p<0.01$) decreases in $\dot{Q}_L$ compared to YW. In addition, OM had less of a decrease ($p=0.01$) in $\dot{Q}_L$ compared to YM. At the final min, OW had greater ($p=0.04$) decreases in $\dot{Q}_L$ compared to YW. From baseline to the final min, LVR increased ($p<0.01$) in all groups. At min 2, LVR increased greater ($p<0.01$) for OW compared to YW. At min 4, OM had greater increases in LVR compared to YM ($p<0.01$) and OW ($p<0.01$). At the final min, OW ($p<0.01$) had greater

increases in LVR compared to YW. OW exhibited greater decreases ($p=0.02$) from baseline to the final min in superficial femoral diameter compared to YW, while not different ($p>0.18$) than YM or OM (data not shown). End-tidal CO_2 was not different across time during the 65% IRBT for YM ($p>0.37$), YW ($p>0.51$), and OW ($p>0.25$). For OM, end-tidal CO_2, compared to baseline, was higher ($p<0.01$) at min 2, but not different at min 4 ($p=0.14$) or the final min ($p=0.36$) (data not shown). During the 65% IRBT, no muscle contraction was measured via EMG.

YM and OM comparisons when matched for PI_{MAX}: Figure 5 shows the change in MAP and percent change in $\dot{Q}_L$ and LVR at the final min of the 65% IRBT in a subset (n=5) of YM and OM matched for PI_{MAX}. PI_{MAX} ($p=0.52$) was not different between YM and OM. At the final min, MAP ($p=0.36$), $\dot{Q}_L$ ($p=0.80$), and LVR ($p=0.83$) were not different between YM and OM.

Discussion

Major findings: This study was designed to determine the influences of age on sex differences in the inspiratory muscle metaboreflex. The major findings of the present study were three-fold. First, in OW compared to YW, the activation of the inspiratory muscle metaboreflex led to greater 1) increases in MAP, LVR, and RPP (an index of myocardial demand) as well as 2) decreases in $\dot{Q}_L$ at the final min. Second, inspiratory muscle metaboreflex-induced cardiovascular consequences were not different at the final min between YM and OM even with matched PI_{MAX}. Lastly, sex differences were not present in the cardiovascular consequences of the inspiratory muscle metaboreflex in older adults. These findings suggest that OW exhibit greater inspiratory muscle metaboreflex-induced cardiovascular consequences compared to YW, while no differences occur with age in men.

Inspiratory muscle metaboreflex: High inspiratory muscle work leads to time-dependent neural and cardiovascular consequences. In support of this, fatiguing diaphragmatic contractions in the anesthetized rat leads to increased type IV (metabosensitive) afferent discharge (21) and stimulation of the phrenic afferents leads to vasoconstriction and decreases in blood flow (23). In the current study, we found high inspiratory muscle work led to increases in MAP and LVR resulting in decreases in $\dot{Q}_L$ in accordance with previous studies (42, 43, 47, 49). Consistent with previous studies (42, 47, 49), we found maintaining the same breathing frequency and duty cycle without the high inspiratory muscle load did not lead to changes in MAP, $\dot{Q}_L$, or LVR, suggesting the high inspiratory muscle work-induced metabolic accumulation is responsible for these cardiovascular responses. It is important to note that metabolite accumulation has been shown to increase Type III (mechanosensitive) afferent activity (44), suggesting that it is possible the mechanoreflex may have contributed to the MAP response during the 65% IRBT.

Other sources such as central command and arterial baroreceptors may also contribute to the neural and cardiovascular responses observed in the current study. However, it has previously been shown that performing the IRBT at near maximal inspiratory pressures (without inducing fatigue) does not elicit increases in MSNA, MAP, LVR, or decreases in $\dot{Q}_L$ until after three min suggesting central command is not contributing to the cardiovascular responses during the 65% IRBT (42, 49).

Effect of age on the inspiratory muscle metaboreflex: Older age is associated with cardiovascular and neural adjustments. For example, resting blood pressure and MSNA are generally higher in older adults compared to their younger counterparts (19). Previous findings regarding the effect of aging on the skeletal muscle metaboreflex have been inconclusive. Specifically, the skeletal muscle metaboreflex has been shown to be attenuated (22, 32, 38), preserved (41), or augmented (7, 34) in older adults. Several potential reasons may explain these inconsistent findings, such as different exercise modalities, not accounting for sex differences, and not matching maximum voluntary contraction (MVC) between groups. For example, sex differences have been reported in the metaboreflex-induced neural and cardiovascular responses in pre-menopausal women compared to young men (11, 16). Furthermore, previous studies that matched MVC have found that the increase in blood pressure, MSNA, and calf/renal vascular resistance were not different between older and younger adults (15, 30, 37, 41, 50).

Older age also is associated with changes within the pulmonary system such as loss of elastic recoil (28) and stiffening of the chest wall (26). Consequently, older age is associated with a higher work and cost of breathing for a given ventilation (26). Furthermore, older adults have less inspiratory muscle fatigue resistance compared to younger adults (5) suggesting that older adults will have a greater inspiratory muscle metaboreflex compared to younger

individuals. In the present study, there was no effect of age on the cardiovascular consequences of the inspiratory muscle metaboreflex in men. Furthermore, no differences were present when PI_{MAX} was matched, consistent with the previous findings investigating the skeletal muscle metaboreflex when MVC was matched between younger and older men (15, 50).

In contrast, we found that the activation of the inspiratory muscle metaboreflex led to greater increases in MAP, LVR, pulse pressure, RPP as well as decreases in $\dot{Q}_L$ in OW compared to YW. What are potential mechanisms for the greater inspiratory muscle metaboreflex-induced cardiovascular consequences in women with age? First, OW demonstrate greater sympathetic vasoconstriction during exercise compared to YW (12). In addition, OW, compared to YW, exhibit more transduction of sympathetic outflow to the peripheral vasculature (20). Both of these mechanisms likely contributed to the greater inspiratory muscle metaboreflex-induced increases in MAP and LVR as well as decreases in $\dot{Q}_L$ in OW compared to YW. Second, older age has been associated with aortic stiffness (36) leading to higher resting systolic and pulse pressures. In addition, the metaboreflex has been shown to increase arterial stiffness, as evident by an augmented femoral-tibial pulse wave velocity, in the non-exercising limb in young individuals (9). Recently, Figueroa et al (2015) found that metaboreflex activation led to a greater augmentation index, indicative of vascular stiffness, in post-menopausal women compared to pre-menopausal women (13). These previous studies in combination with the current findings suggest that inspiratory muscle metaboreflex activation led to greater arterial stiffness in OW compared YW subsequently leading to greater increases in pulse pressure and RPP. Third, the arterial baroreceptors interact with the skeletal muscle metaboreflex for control of neural and cardiovascular changes (8, 24). Although the neural interaction between the arterial baroreceptors and skeletal muscle metaboreflex is not modulated in men with age (15), it

is possible this neural interaction is altered in OW compared to YW. It is currently not known if the arterial baroreceptors interact with the inspiratory muscle metaboreflex. Future studies are required to determine if the neural interaction between the arterial baroreceptors and inspiratory muscle metaboreflex is altered with age in men and women. Collectively, our data suggests that inspiratory muscle metaboreflex activation during severe-intensity exercise in women may lead to greater redistribution of $\dot{Q}_L$ from the locomotor muscles to the inspiratory muscles in OW compared to YW. In addition, the higher RPP in OW with the activation of the inspiratory muscle metaboreflex has implications in clinical and subclinical populations. For example, the higher metaboreflex-induced RPP and associated myocardial $\dot{V}O_2$ and blood flow (14, 27) may result in earlier onset of angina for a given exercise workload (39).

Limitations: Several potential limitations may have influenced our results. First diaphragmatic fatigue was not directly assessed in the current study. This protocol (i.e. 65% PI_{MAX}, breathing frequency, duty cycle) has previously been shown to elicit diaphragmatic fatigue in younger individuals (42). However, it is currently unknown if the protocol elicited greater development of diaphragmatic fatigue in the older adults compared to the younger adults. Second, inspiratory mouth pressure was not directly measured during the IRBTs. However, the magnitudes of change of MAP, $\dot{Q}_L$, and LVR in the young adults of the current study are in excellent agreement with previous studies (42, 43, 49) suggesting the protocol was sufficient to activate the inspiratory muscle metaboreflex. Lastly, measurements of sympathetic activation would have provided additional valuable information regarding underlying mechanisms responsible for the differences observed between OW and YW.

Conclusion: Our cross sectional data suggest that older age leads to greater inspiratory muscle metaboreflex-induced cardiovascular consequences and consequently greater work of the heart in

women, but not men. Furthermore, sex differences in the cardiovascular consequences of the inspiratory muscle metaboreflex are not present in older adults. Future studies should examine if baroreflex sensitivity with metaboreflex activation is altered in women across age and if age influences the degree of exercise-induced diaphragmatic fatigue development.

Table 4-1 Subject Characteristics

	YM	OM	OW	YW
n	8	8	8	8
Age (yrs)	22 ± 2	67 ± 6*	64 ± 4*	22 ± 2
Ht (cm)	179 ± 7	174 ± 8†	165 ± 4	163 ± 4
Wt (kg)	77 ± 8	80 ± 12	72 ± 7	63 ± 12
SBP (mmHg)	124 ± 6	129 ± 14	120 ± 10	111 ± 9
DBP (mmHg)	75 ± 6	83 ± 10	77 ± 10	70 ± 5
MAP (mmHg)	91 ± 5	98 ± 11	91 ± 9	83 ± 6
Pulse pressure (mmHg)	49 ± 7	46 ± 5	44 ± 8	41 ± 7
RPP (bpm*mmHg)	85 ± 17	87 ± 15	90 ± 24	74 ± 15
FVC (L)	5.8 ± 0.5	4.7 ± 1*†	3.5 ± 0.4	4.0 ± 0.5
FVC (% predicted)	110 ± 6	121 ± 19	115 ± 11	108 ± 10
FEV_1 (L)	4.7 ± 0.6	3.5 ± 0.6*†	2.6 ± 0.3*	3.3 ± 0.3
FEV_1 (%predicted)	104 ± 10	115 ± 16	107 ± 13	103 ± 8
PEF ($L\ s^{-1}$)	10.1 ± 1.9	9.0 ± 1.5†	6.2 ± 1.1	6.4 ± 0.9
PEF (%predicted)	106 ± 22	108 ± 14	108 ± 20	94 ± 13
PI_{MAX} (cmH_20)	161 ± 39	119 ± 23*	90 ± 31	124 ± 34
PI_{MAX} (%predicted)	130 ± 37	109 ± 23	131 ± 42	138 ± 33

Mean±SD. YM, young men; OM, older men; OW, older women; YW, younger women; Ht, height; Wt, weight; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; RPP, rate pressure product; FVC, forced vital capacity; FEV_1, forced expiratory volume in 1 s; PEF, peak expiratory flow; PI_{MAX}, maximal inspiratory mouth pressure; *, different ($p<0.05$) from younger counterpart; †, different ($p<0.05$) from OW

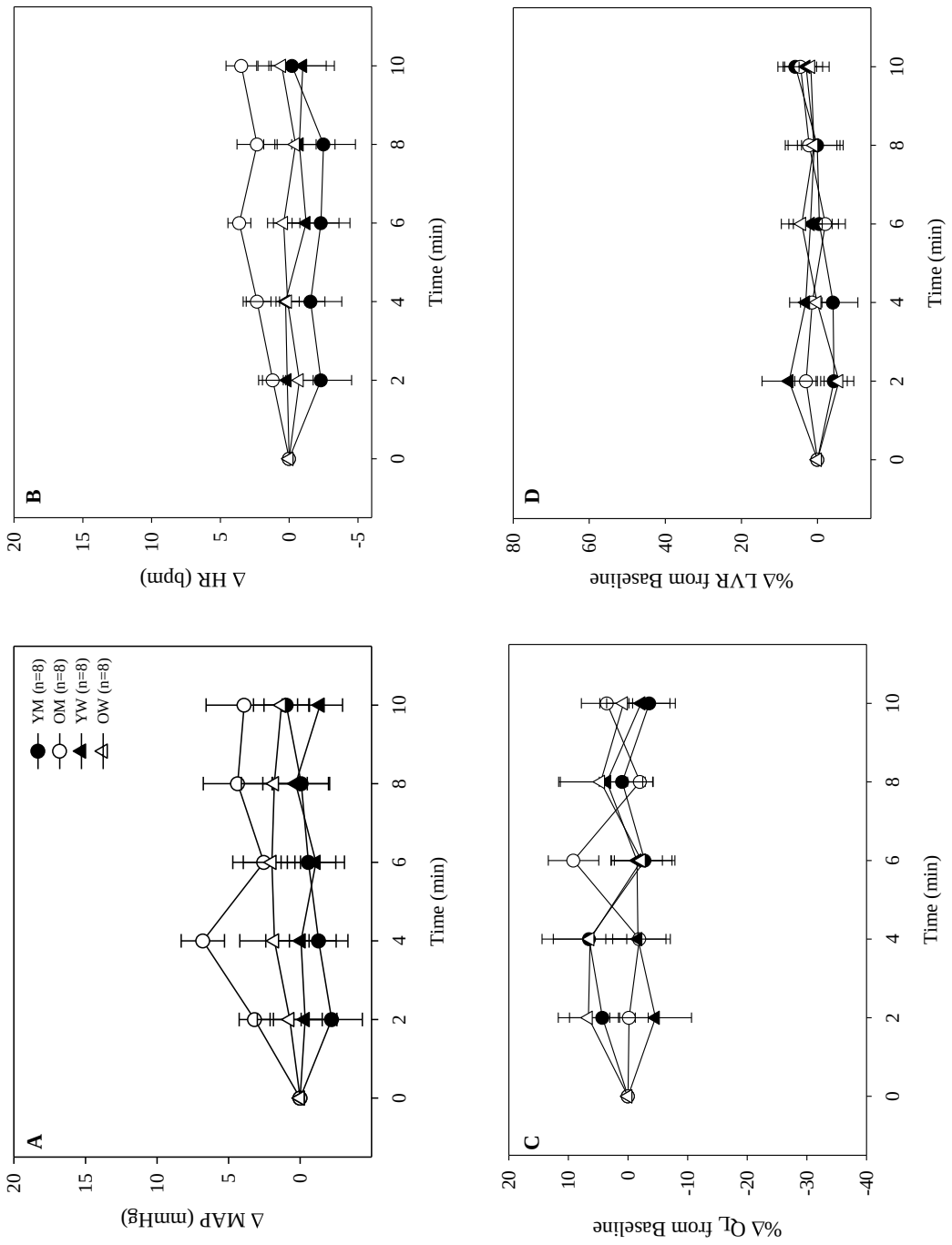

Figure 4-1 Cardiovascular responses during the 2% IRBT.

The change from baseline for MAP (A) and HR (B) and % change from baseline for $\dot{Q}_L$ (C) and LVR (D) for YM (closed circles), OM (open circles), YW (closed triangles), and OW (open triangles) during the 2% IRBT. There were no significant differences among groups (all $p>0.23$) or across time (all $p>0.27$) for any of the cardiovascular responses.

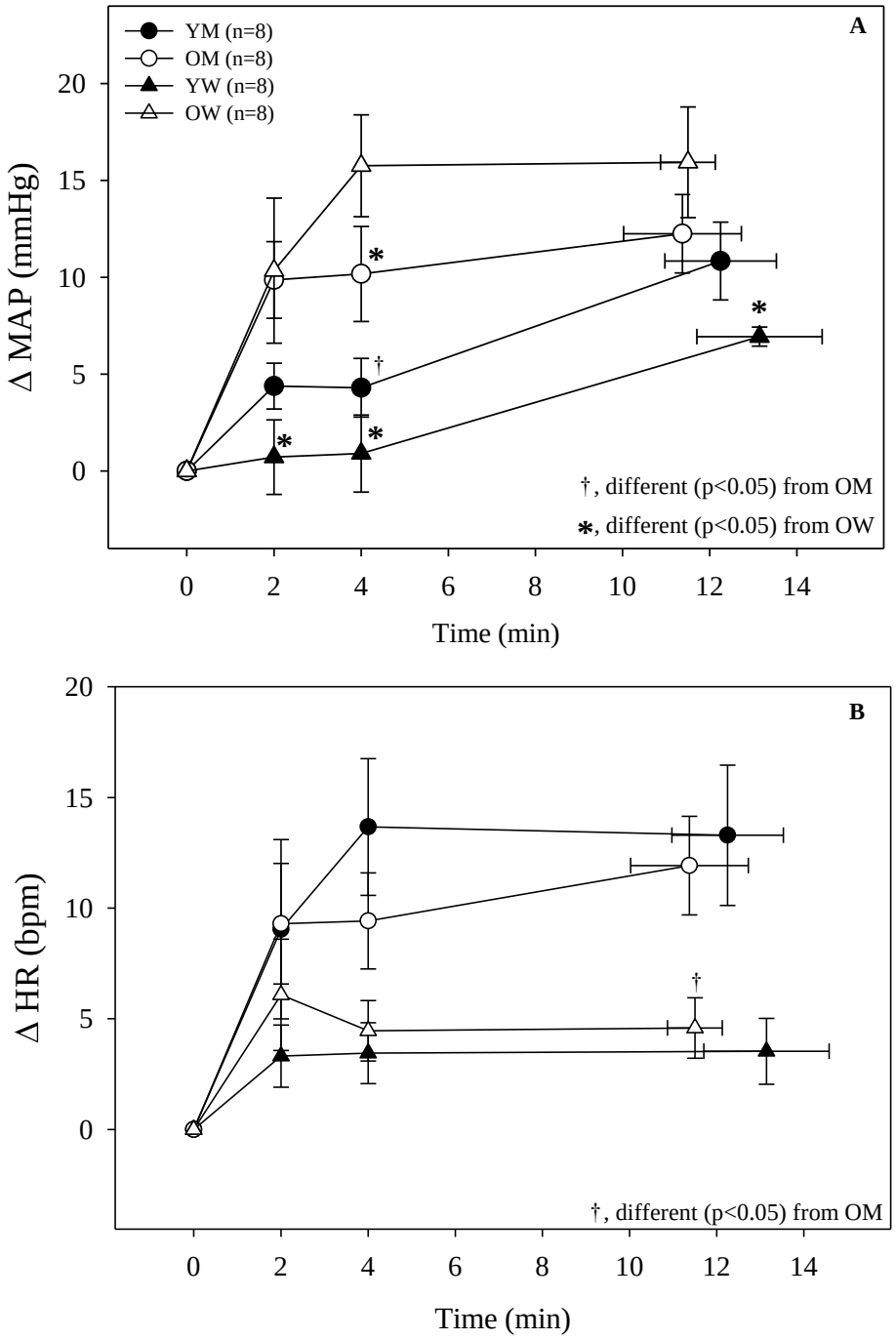

Figure 4-2 MAP and HR responses during the 65% IRBT.

The change from baseline for MAP (A) and HR (B) for YM (closed circles), OM (open circles), YW (closed triangles), and OW (open triangles) during the 65% IRBT. At the final min, OW had a greater ($p<0.01$) increase in MAP compared to YW. OM had significantly greater increases in HR at the final min compared to OW.

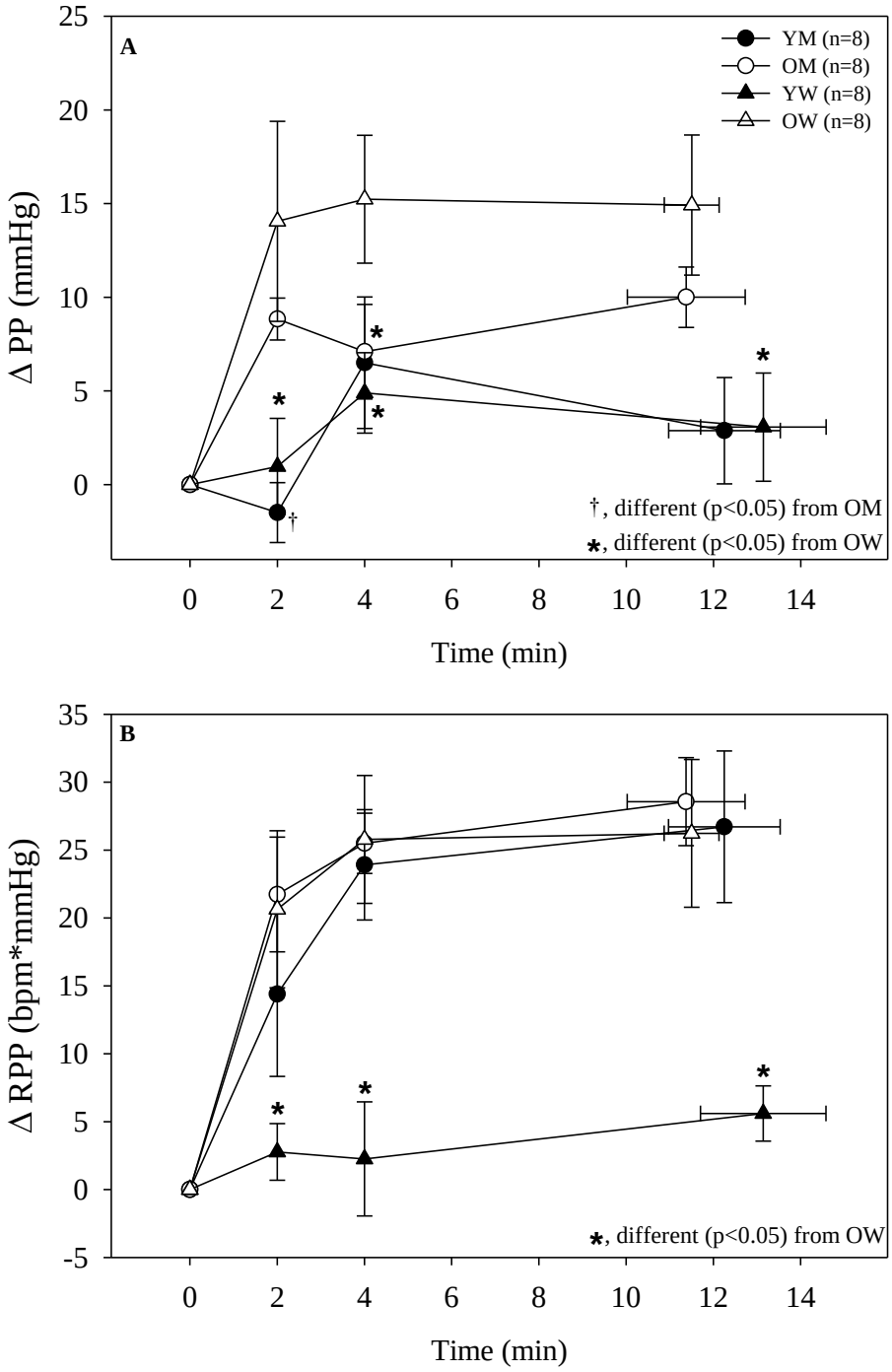

Figure 4-3 Pulse pressure and RPP during the 65% IRBT.

The change from baseline for pulse pressure (A) and RPP (B) for YM (closed circles), OM (open circles), YW (closed triangles), and OW (open triangles) during the 65% IRBT. At the final min, OW had greater increases ($p<0.01$) in pulse pressure than YW. The increase in RPP was greater for OW ($p<0.01$) compared to YW at the final min.

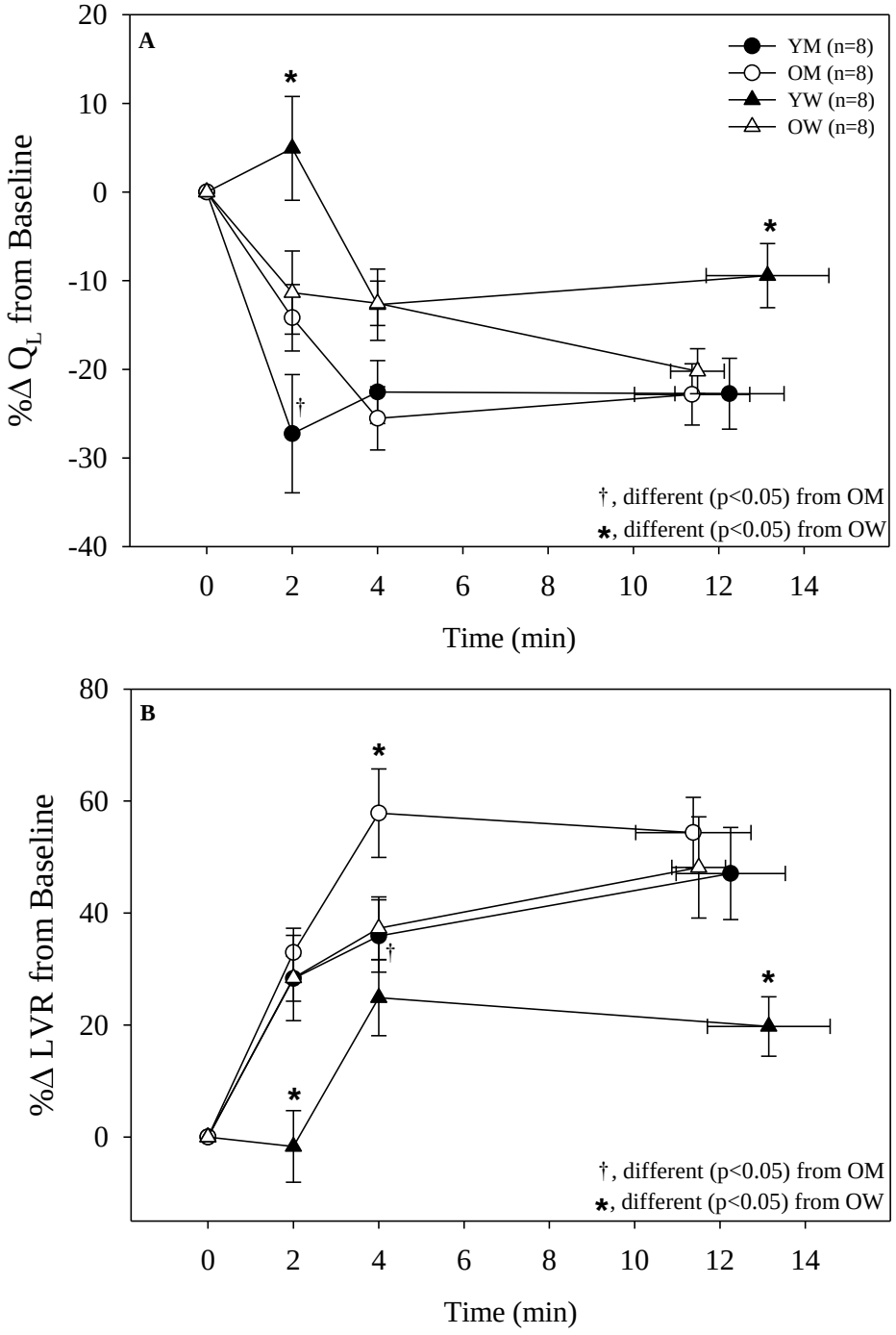

Figure 4-4 $\dot{Q}_L$ and LVR responses during the 65% IRBT.

The percent change from baseline for $\dot{Q}_L$ (A) and LVR (B) for YM (closed circles), OM (open circles), YW (closed triangles), and OW (open triangles) during the 65% IRBT. At the final min, OW had greater ($p=0.04$) decreases in $\dot{Q}_L$ compared to YW. At the final min, OW ($p<0.01$) had greater increases in LVR compared to YW.

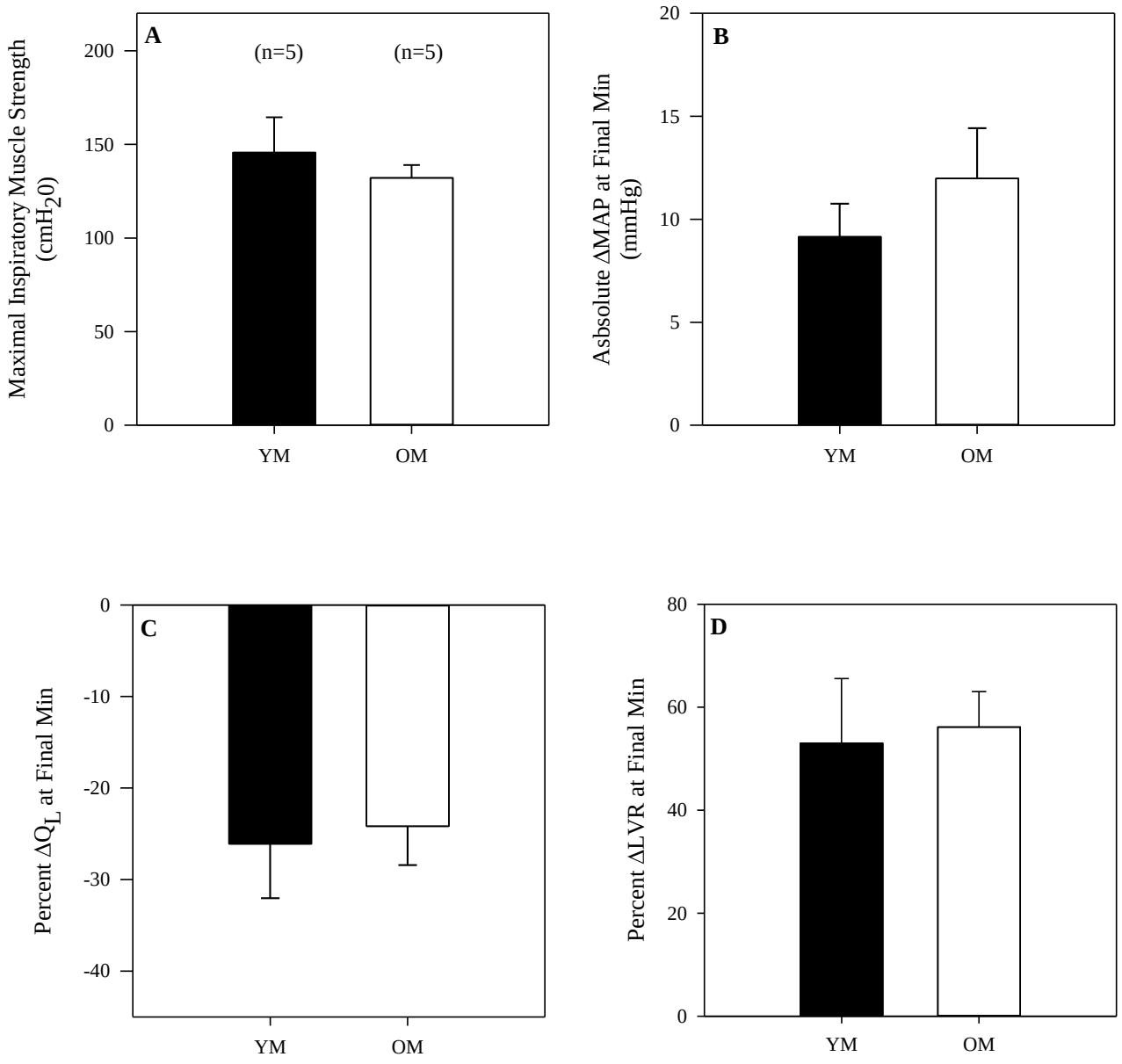

Figure 4-5 Cardiovascular comparisons between YM and OM when PI_{MAX} was matched. Changes in MAP, $\dot{Q}_L$, and LVR between YM and OM when matched for PI_{MAX}. The increases in MAP (p=0.36) and LVR (p=0.83) and decreases in $\dot{Q}_L$ (p=0.80) were not different between YM and OM when PI_{MAX} were not different (p=0.52).

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Chapter 5 - Conclusion

Integrating the investigations described in this dissertation, sex differences between young men and women are concluded not to influence respiratory muscle blood flow regulation, but have a substantial impact on the inspiratory muscle metaboreflex. Specifically, our data demonstrated that respiratory muscle (diaphragm, intercostal, and transversus abdominis) blood flow and vascular conductance were not different between males and females at rest or during moderate and near-maximal intensity exercise. However, sex differences were present in the inspiratory muscle metaboreflex. Specifically, pre-menopausal women, compared to age-matched men, had attenuated changes in mean arterial pressure (MAP), limb blood flow ($\dot{Q}_L$), and limb vascular resistance (LVR) with inspiratory muscle metaboreflex activation. When we examined if sex differences were present in older adults, we found post-menopausal women, compared to pre-menopausal, had greater changes in MAP, $\dot{Q}_L$, and LVR with activation of the inspiratory muscle metaboreflex such that sex differences were no longer present between older men and women. Taken together, these studies suggest that in populations with high work of breathing (e.g. chronic heart failure), the tonically active inspiratory muscle metaboreflex will redistribute less $\dot{Q}_L$ from the locomotor muscles to the respiratory muscles in pre-menopausal women compared to men resulting in less exercise tolerance impairments; while these sex differences are no longer present in older adults.

Appendix A - CURRICULUM VITAE

JOSHUA R. SMITH

University Education:

Kansas State University	Department of Kinesiology	Ph.D.
Manhattan, KS	Exercise Physiology	
2013-2017	Advisor: Craig A. Harms, Ph.D.	

Dissertation: Sex Differences in the Cardiopulmonary Responses to Exercise

Kansas State University	Department of Kinesiology	M.S.
Manhattan, KS	Exercise Physiology	
2011-2013	Advisor: Craig A. Harms, Ph.D.	

Thesis: The Influence of Respiratory Muscle Fatigue on Inactive Blood Flow

Indiana University	Department of Biology	B.S.
2007-2011	Biology	

Teaching

Kansas State University	Department of Kinesiology		
Manhattan, KS	2011-2012	Activity/Lab courses	Graduate Assistant
	2012-2016	Exercise Physiology Lab	Graduate Assistant
	2013-2017	Anatomy &Physiology Lab	Graduate Assistant
	2013-2016	Exercise Physiology Lab	Lab Manager
Manhattan Area Technical Institute			
Manhattan, KS	Fall 2012	Anatomy and Physiology	Instructor
Cloud Community College			
Junction City, KS	Spring 2013	General Biology	Instructor
Highland Community College			
Wamego, KS	Spring 2014	Nutrition	Instructor
Highland Community College			
Wamego, KS	Summer 2016	General Biology	Instructor

Service:

- Kansas State University Kinesiology Student Association Speaker 2013, 2015
- Department of Kinesiology Faculty Search Committee Member 2015
- Judge for Human Ecology Undergraduate Research Forum 2015, 2017
- Human College Graduate Forum Poster Peer Reviewer 2016

Organizations
American College of Sports Medicine, 2011-
American Physiological Society, 2013-
American Heart Association, 2015-

Publications
Original Research

21. Smith JR, Alexander AM, Hammer SM, Didier KD, Kurti SP, Broxterman RM, Barstow TJ, Harms CA. Cardiovascular Consequences of the Inspiratory Muscle Metaboreflex: Effects of Age and Sex. *AJP Heart Circ Physiol* doi: 10.1152/ajpheart.00818.2016.

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12. Skutnik BC, Smith JR, Johnson AM, Kurti SP, Harms CA. The Effect of Low-Volume Interval Training on Resting Mean Arterial Pressure in Pre-hypertensive Subjects: A Preliminary Study. *Phys Sportsmed* 44(2):177-183, 2016.

11. Johnson AM, Kurti SP, Smith JR, Rosenkranz SR, Harms CA. Effects of an Acute Bout of Moderate Intensity Exercise on Airway Inflammation and Postprandial Lipemia. *Appl Physiol Nutr Metab* 41(3):284-291, 2016.

10. Kurti SP, Murphy JD, Ferguson C.S., Brown KB, Smith JR, Harms CA. Improved Lung Function following Dietary Antioxidant Supplementation in Exercise-induced Asthmatics. *Resp Phys Neurobiol* 220:95-101, 2016.

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8. Broxterman RM, Craig JC, Smith JR, Wilcox SL, Chen J, Warren S, Barstow TJ. Influence of Blood Flow Occlusion on the Development of Peripheral and Central Fatigue during Small Muscle Mass Handgrip Exercise. *J Physiol* 593(17):4043-4053, 2015.

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Manuscripts in Review

Smith JR, Didier KD, Hammer SM, Alexander AM, Kurti SP, Copp SW, Barstow TJ, Harms CA. Effect of Cyclooxygenase Inhibition on the Inspiratory Muscle Metaboreflex-Induced Cardiovascular Consequences. *In Revision*

Smith JR, Hagemen KS, Poole DC, Harms CA, Musch TI. Effect of Chronic Heart Failure in Older Rats on Respiratory Muscle and Hindlimb Blood Flow during Submaximal Exercise. *In Review*

Smith JR, Sutterfield SL, Baumfalk DR, Didier KD, Hammer SM, Ade CJ. Left Ventricular Strain is Reduced during Voluntary Apnea in Healthy Humans. *In Review*

Manuscripts in Preparation

Alexander AM, Smith JR, Emerson SR, Kurti SP, Barstow TJ, Harms CA. Effect of puberty on gas exchange threshold in boys and girls. *In Preparation*

Kurti SP, Smith JR, Rosenkranz SK, Jurrens K, Laughlin A, Harms CA. Deep Inspirations Modify Postprandial Airway Inflammation in Non-asthmatic Subjects: A Randomized Crossover Study. *In Preparation*

Smith JR, Ferguson SK, Harms CA, Musch TI, Poole DC. Does Beetroot Juice Supplementation Ameliorate the Elevated Diaphragm Blood Flow in Chronic Heart Failure during Submaximal Exercise? *In Preparation*

Smith JR, Hagemen KS, Poole DC, Harms CA, Musch TI. Respiratory Muscle Blood Flow during Submaximal Exercise in Older and Younger Rats. *In Preparation*

Book Chapters

Harms CA, Smith JR, Kurti SP. Sex Differences in Normal Pulmonary Structure and Function at Rest and during Exercise. In: Hemnes AR, editor. Gender, Sex Hormones, and Respiratory Disease. Springer International Publishing; 2016, p.1-26.

Viewpoints:

1. Harms CA & Smith JR. When would Performance Improve with Inspiratory Muscle Training? *J Physiol*. 2012.

2. Smith JR & Copp SW. Comment on Viewpoint Manuscript: Could small-diameter muscle afferents be responsible for the ergogenic effect of limb ischemic preconditioning? *J Appl Physiol* 2017.

Abstracts:

2017

Smith JR, Didier KD, Hammer SM, Alexander AM, Kurti SP, Barstow TJ, Harms CA. Contribution of Prostaglandins to the Inspiratory Muscle Metaboreflex-Induced Cardiovascular Consequences. Experimental Biology. Chicago, IL (Poster).

Smith JR, Alexander AM, Hammer SM, Didier KD, Kurti SP, Broxterman RM, Barstow TJ, Harms CA. Effect of Aging on Sex Differences in the Inspiratory Muscle Metaboreflex. American College of Sports Medicine International Conference. Denver, CO (Poster).

Stein JA, Smith JR, Ade CJ, Broxterman RM, Sanborn B, Barstow TJ, Heinrich KM. Oxygen Uptake during Three Varying Duration High-Intensity Functional Training Sessions. American College of Sports Medicine International Conference. Denver, CO (Poster).

2016

Smith JR, Broxterman RM, Hammer SM, Alexander AM, Didier KD, Barstow TJ, Kurti SP, Harms CA. Effect of Aging on the Inspiratory Muscle Metaboreflex. Experimental Biology Conference. San Diego, CA (Poster).

Smith JR, Broxterman RM, Hammer SM, Alexander AM, Didier KD, Barstow TJ, Kurti SP, Harms CA. Sex Differences in the Inspiratory Muscle Metaboreflex. American College of Sports Medicine International Conference. Boston, MA (Oral Presentation).

Kurti SP, Emerson SR, Rosenrkanz SK, Teeman CS, Emerson EE, Cull BJ, Smith JR, Harms CA. Post-prandial exhaled 8-isoprostane responses to meals of varying caloric and fat content in non-asthmatic, insufficiently active men. Experimental Biology Conference. San Diego, CA (Poster).

Kurti SP, Smith JR, Rosenkranz SK, Jurrens K, Laughlin A, Harms CA. Deep Inspirations Attenuate Postprandial Airway Inflammation in Non-Asthmatic Adults: A Randomized Crossover Study**.** American College of Sports Medicine International Conference. Boston, MA (Poster).

2015

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Evans KK, Smith JR, Harms CA. The Effect of Acute Vitamin C Supplementation on Airway Function during Exercise in Exercise-induced Asthmatics. Kansas State University Developing Scholars Program Research Poster Symposium. Manhattan, KS (Poster).

Smith JR, Johnson AM, Kurti SP, Kolmer SA, Harms CA. Impact of Physical Activity on Airway Responsiveness and Bronchodilation in Healthy Subjects. American College of Sports Medicine International Conference. San Diego, CA (Oral Presentation).

Smith JR, Broxterman RM, Ade CA, Barstow TJ, Harms CA. The Effect of *N*-acetylcysteine on peripheral hemodynamics and fatigue during exercise. Experimental Biology Conference. Boston, MA (Poster).

2014

Smith JR, Johnson AM, Kurti SP, Kolmer SA, Harms CA. Impact of Physical Activity on Airway Responsiveness and Bronchodilation in Healthy Subjects. ACSM Conference on Integrative Physiology of Exercise. Miami Beach, FL (Poster).

Smith JR, Emerson SR, Harms CA. Airways and Lung Growth from Pre- to Post-Puberty. American College of Sports Medicine International Conference. Orlando, FL (Poster).

Kurti SP, Emerson SR, Smith JR, Castinado KM, Harms CA. The Effect of an High Intensity Interval Training Session on Respiratory Muscle Fatigue. American College of Sports Medicine International Conference. Orlando, FL (Poster).

Johnson AM, Kurti SP, Smith JR, Rosenkranz SK, Harms CA. Effects of an Acute Bout of Moderate Intensity Exercise on Airway Inflammation and Postprandial Lipemia. American College of Sports Medicine International Conference. Orlando, FL (Poster).

Emerson SR, Kurti SP, Rosenkranz SK, Smith JR, Harms CA. Sex Differences in Cardiopulmonary Function during Exercise from Pre- to Post-Puberty. American College of Sports Medicine International Conference. Orlando, FL (Poster).

2013

Smith JR, Emerson SR, Harms CA. Airways and Lung Growth from Pre- to Post-Puberty. American College of Sports Medicine Central States Conference. Warrensburg, MO (Poster).

Kurti, SP, Emerson SR, Smith JR, Castinado KM, Harms CA. The Effect of an High Intensity Interval Training Session on Respiratory Muscle Fatigue. American College of Sports Medicine Central States Conference. Warrensburg, MO (Poster).

Smith JR, Ade CJ, Broxterman RM, Skutnik BC, Harms CA. Effect of respiratory muscle fatigue on inactive arm blood flow during cycling exercise. American College of Sports Medicine International Conference. Indianapolis, IN (Poster).

Skutnik, BC, Smith JR, Johnson AM, Harms CA. The effects of High Intensity Training on Mean Arterial Pressure and C-reactive Protein. American College of Sports Medicine International Conference. Indianapolis, IN (Poster).

2012

Smith JR and Harms CA. Prevalence and determination of expiratory flow limitation during exercise in men and women. American College of Sports Medicine International Conference. San Francisco, CA (Poster).

Grants:

- 2012 Kansas State University Graduate School Travel Grant ($75)
- 2013 Kansas State University Graduate School Travel Grant ($400)
- 2014-7 Kansas State University Graduate School Travel Grant ($500)
- American Heart Association Midwest Affiliate Predoctoral Fellowship "Sex differences in the Cardiovascular and Fatigue Consequences of Inspiratory Muscle Fatigue" Unfunded
- 2015 K-State Human Ecology Doctoral Dissertation Research Award ($1,000) Funded
- 2016 ACSM Foundation Doctoral Student Research Grant "The Effect of Aging on the Inspiratory Muscle Metaboreflex" Funded ($5,000)

Awards:

- 2013 American College of Sports Medicine Featured Poster Award for Basic Science in the Cardiovascular, Renal, and Respiratory section at annual meeting
- 2014 Joint Commission on Sports Medicine and Science second runner up Graduate Fellow ($500)
- 2015 Kinesiology Department Outstanding Doctoral Student Award ($750)
- 2016 American Kinesiology Association Doctoral Student of the Year Award
- 2016 Kansas State University Golden Key GTA of the Year
- 2017 ACSM Charles M. Tipton Student Research Award

Reviewer:

European Journal of Applied Physiology
Experimental Physiology
Journal of Applied Physiology
Journal of Sports Sciences
Respiratory Physiology and Neurobiology